Case 4-18634/A/CCN

#28
JRP
2/9/95

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE US PATENT APPLICATION OF
YIQING ZOU ET AL.
SERIAL NO. 08/216,440
FILED: March 23, 1994
FOR: ANTIMALARIAL COMPOSITIONS

Commissioner of Patents and Trademarks
Washington D.C. 20231 USA

DECLARATION OF WALTHER H. WERNSDORFER UNDER RULE 132

I, Walther H. Wernsdorfer, citizen of the Federal Republic of Germany and resident of Vienna, Austria, do hereby declare and say as follows:

That I am a Graduate of The Friedrich Alexander University of Erlangen, Federal Republic of Germany, where I graduated in 1952 and obtained the approbation in medicine (M.B.B.S);

That I am a Graduate of The Ludwig Maximilian University of Munich, Federal Republic of Germany, where I graduated in 1953 and obtained the Degree of a Doctor of Medicine (M.D.);

That I have undergone postgraduate training in tropical medicine at the Swiss Tropical Institute in Basel, Switzerland, and obtained in 1952 the Diploma of Tropical Medicine (D.T.M.);

That I have undergone postgraduate training in public health at the University of Bristol, U.K., and obtained in 1967 the Diploma of Public Health (D.P.H.);

That, as from 1958 until 1988, I have served the World Health Organization as a staff member in the fields of tropical medicine and malaria; between 1978 and 1988 as Chief Medical Officer in charge of global malaria research and *ex officio* Secretary of the Scientific Working Groups on the Chemotherapy and Immunology of Malaria, UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases;

That, as from 1960, I held academic teaching assignments in addition to my WHO assignments, with the Faculty of Medicine, University of Khartoum, Sudan, the University of Tunisia, and the Université Claude Bernard, Lyon, France;

That, in 1988, I have been appointed visiting professor at the University of Vienna, Austria, and the Universiti Sains Malaysia, Penang, and in 1993 at the Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand;

That I am the principal author or coauthor of approximately 100 publications, mainly in the field of malaria and malaria chemotherapy;

That I am a registered member of the medical profession (Medical Board of Central Franconia, Federal Republic of Germany);

That I am a member of the following professional bodies/organizations:

World Health Organization (WHO) Expert Panel on Malaria
German Society of Tropical Medicine (Honorary Member)
Swiss Society of Tropical Medicine and Parasitology (Honorary Member)
Austrian Society of Tropical Medicine and Parasitology (Council Member)
Royal Society of Tropical Medicine and Hygiene (U.K.)
British Society of Public Health
British Society of Parasitology;

That I am presently working as Visiting Professor (Tropical Medicine) at the Institute for Specific Prophylaxis and Tropical Medicine, Faculty of Medicine, University of Vienna, Austria, and Visiting Professor at the National Centre for Drug Research, Universiti Sains Malaysia, Penang, Malaysia (Tropical Clinical Pharmacology), and as Visiting Professor at the Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand (Tropical Clinical Pharmacology);

That I am the editor of the standard textbook entitled ***Malaria. Principles and Practice of Malariology*** (ISBN 0 443 024170) with Sir Ian McGregor being the coeditor;

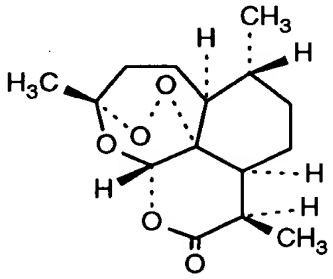
That this textbook was first published in 1988 in two volumes by Churchill Livingstone (Edinburgh, London, Melbourne and New York);

That I am the author of Chapter 51 (Exhibit 1): Recent progress of malaria research: chemotherapy, pages 1569-1674, with P.I.Trigg being the coauthor;

That this chapter and the references cited therein define the complete state of the art in 1988 with regards to the natural product qinghaosu (artemisinin) and the derivatives synthesized therefrom, and also define the relevant state of the art at the first U.S. filing date of Ser.No. 07/714,229, filed June 12, 1991;

That the following statements and the conclusion drawn therefrom agree with the contents of this Chapter 51 from the textbook;

That at this first U.S. filing date the antimalarial activity against *Plasmodium falciparum* of the natural antimalarial product qinghaosu (=artemisinin), isolated from the indigenous plant Qinghao (*Artemisia annua* L.), was established in various *in-vitro* and *in-vivo* experimental models and in clinical trials involving patients with naturally acquired malaria:

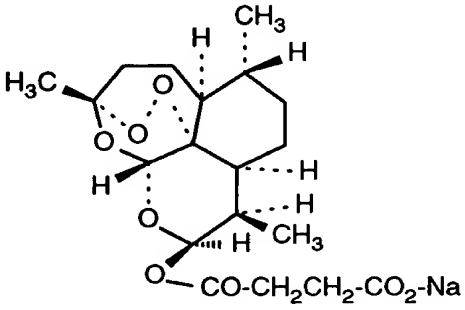
I:  (qinghaosu, artemisinin),

That the reason for preparing structural derivatives of qinghaosu appears its insufficient ability to effect complete parasite clearance, which has been established in clinical trials in which this natural product was tested by administering different dosage forms such as tablets, capsules or intramuscular injections, thus rendering qinghaosu unsatisfactory for the radical treatment of malaria;

That the unsatisfactory curative effect of qinghaosu was ascribed to its poor solubility in water and injectible carriers precluding the establishment of adequate concentrations in the blood;

Water soluble artemisinin derivative

That at this first U.S. filing date, the following structural derivatives of artemisinin (Ia, Ib) had been selected for inclusion in pharmaceutical dosage forms suitable for human administration:

Ia:  (Na-artesunate, i.v.,i.m., water solution);

That Na-artesunate is characterized by a grossly improved water solubility as compared to qinghaosu;

That Na-artesunate is higly unstable in water, leading by hydrolysis within less than one hour to a nearly complete transformation to dihydro-artemisinin and Na-succinate;

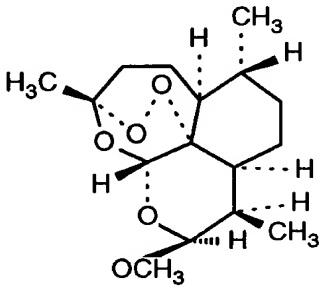
That the improved water solubility of Na-artesunate is attributed to a higher degree of hydrophility as compared to qinghaosu resulting from the substitution of the qinghaosu structure with the hydrophilic Na-succinyl group;

That the improved water solubility of Na-artesunate and subsequent formation of dihydro-artemisinin resulted in an increased antimalarial activity upon oral administration as compared to artemisinin due to increased absorption in the gastro-intestinal tract;

That due to this increased gastro-intestinal absorption Na-artesunate appeared suitable for oral administration;

That due to the low stability in aqueous suspension and its hydrolysis product dihydroartemisinin, as unanimously reported in various references, Na-artesunate appeared unsuitable for formulation in an oral dosage form with a stability acceptable to regulatory authorities;

Water insoluble artemisinin derivative

Ib:  (artemether, i.m., oily solution);

That artemether is characterized by an extremely low water solubility similar to qinghaosu but satisfactory solubility in unpolar carrier liquids such as oils;

That the low water solubility and the hydrophobic character of artemether is self-evident from the absence of a hydrophilic group in the structure;

That due to the solubility of artemether in organic carrier liquids such as oils this hydrophobic derivative appeared particularly suitable for the preparation of intramuscular dosage forms containing pharmaceutically acceptable oils as carrier liquids;

That the absorption of artemether from the gastro-intestinal tract was *a priori* thought to be poor due to the relatively high stability and the hydrophobic character of this natural product;

That the oral route of administration of the individual agent artemether was deemed blocked until 1992 as clearly stated by J.Karbwang et al., The Lancet, Vol. 340, Nov. 21, 1992 (1245-1247): Comparison of oral artemether and mefloquine in acute uncomplicated falciparum malaria (Exhibit 2):

Artemether is an effective antimalarial drug with a rapid onset of action that destroys asexual parasites at an early stage of development. The potency of this drug has been shown in clinical trials in China and Burma (references omitted). Artemether clears parasites rapidly with virtually no side-effects. Artemether is, however, associated with a high rate of recrudescence that varies with duration of treatment and the total dose given. The recommended dose of artemether has been 600 mg given over 5 days, but we have found the cure rate to be only 90 % with intramuscular artemether at this dose (reference omitted). There are no reports on the efficacy of oral artemether in multiple-drug resistant falciparum malaria, and the proper dosage regimen of artemether for the treatment of this condition remains to be decided...

Conclusion

That at the first U.S. filing date a qinghaosu derivative meeting both essential requirements of acceptable stability and solubility was not available for oral administration;

That at the first U.S. filing date the suitability of artemether for inclusion in solid oral dosage forms was not known;

That at the first U.S. filing date the antimalarial effect of a solid oral dosage form containing the combined agents artemether and benflumetol was unpredictable;

That it was unpredictable from the literature that the combination of benflumetol with artemether in the combined oral dosage form would render the insoluble compound artemether water soluble or absorbable in the gastro-intestinal tract;

That it was also unpredictable that a combined dosage form would increase the solubility or the gastro-intestinal absorption of the component artemether;

The Undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issueing thereon.

The following exhibits are part of the Declaration:

1. ***Malaria. Principles and Practice of Malariology***, Chapter 51: Recent progress of malaria research: chemotherapy, pages 1569-1674.

2. J.Karbwang et al., The Lancet, Vol. 340, Nov. 21, 1992 (1245-1247): Comparison of oral artemether and mefloquine in acute uncomplicated falciparum malaria.

Signed at Vienna, Austria

this Seventeenth day of December 1994

Walter Helmut Wernsdorfer

Recent progress of malaria research: chemotherapy

Mechanism of action of antimalarial drugs
Folate pathway antagonists
— Sulfonamides and sulfones
— Dihydrofolate reductase inhibitors
Chloroquine and related blood schizontocides
Antibiotics
Qinghaosu, its derivatives and other plant-derived products
Naphthoquinones
Tissue schizontocides

Mechanisms of drug resistance
Resistance to dihydrofolate reductase inhibitors
Resistance to sulfonamides and sulfones
Resistance to 4-aminoquinolines
Resistance to quinine and mefloquine
Resistance to Qinghaosu and its derivatives
Resistance to antibiotics
Resistance to primaquine

Pharmacokinetics
4-aminoquinolines
Primaquine
Quinine
Proguanil
Sulfadoxine and pyrimethamine
Mefloquine

Repository drugs
Inherently long-acting formulations
Chemical modification of drugs to extend duration of action
Delayed degradation and excretion of antimalarial drugs
Sustained release formulations
Screens for repository formulations

Targeting of drugs

Chemotherapeutic approaches based on parasite biochemistry
Biochemical targets
— Energy metabolism
— Protein synthesis
— Nucleic acid synthesis
— Folate metabolism
— Lipid biosynthesis
Microtubules
Parasite invasion of red cells
Oxidant killing of malaria parasites

Exploitation of potential biochemical targets for drug action

New candidate antimalarials
Candidate antimalarials in an advanced state
— 9-phenanthrenemethanols
— Sesquiterpene lactones
— Pyronaridine
— Enpiroline
Candidate antimalarials in an advanced preclinical state
— 4-aminoquinolines and Mannich bases
— 8-aminoquinolines
— 4-quinolinemethanols
— Quinolones
— Naphthoquinones
— Quinazolines
— Dihydrotriazines
Other compounds of interest

gagement in this field has recently induced several pharmaceutical industrial firms to resume malaria research.

As compared to the classical days of chemotherapeutic research, antimalarial drug development has become a highly complicated procedure. However, the introduction of rational animal models for the primary and secondary screening of candidate compounds (Schmidt 1978; Peters 1980; Schofield et al 1981; Howells 1982; Gwadz et al 1983) has facilitated the identification of suitable candidate compounds. The major expansion of the drug testing procedure took place in the fields of preclinical toxicology, carcinogenicity, teratogenicity and mutagenicity, and clinical pharmacology (WHO 1973; Canfield & Rozman 1974), since the current drug registration procedures demand a very detailed documentation of candidate compounds. As a result, one is often confronted with a paradoxical situation in as much as little information on the toxicology and pharmacokinetics may be available for widely used drugs such as chloroquine, as compared to new compounds, e.g. mefloquine. For the same reasons, the development of drug assay and galenical techniques is more advanced for new drugs.

This, retrospectively incomplete, preclinical and clinical development of the earlier drugs may have led to a suboptimal use of these compounds. Chemotherapeutic research should, therefore, also pay attention to improving the efficacy of the existing, registered compounds. Studies such as those of Gaudette & Coatney (1961) on the possible augmentation of plasma levels and efficacy of chloroquine have pointed in the right direction but were unfortunately not followed up.

There are many examples of ill-placed dogmatism in malaria chemotherapy which may also have contributed to holding up progress in this field. For example, the use of quinine was nearly banned following the introduction of chloroquine, although its quick action makes it an essential drug for the treatment of severe falciparum malaria even that due to chloroquine-sensitive parasites. Similarly, the popular concept that all 4-aminoquinolines resemble each other in their activity and efficacy now appears to be outdated given the comparative studies of chloroquine and amodiaquine, and even more so the recent investigations on new members of this group of compounds.

In this chapter, it is intended to review recent research on both the mechanism of action of antimalarial drugs and that of resistance, the development of new medicaments and some findings related to the classical antimalarials. Pharmacokinetic terms are used in accordance with the Manual of Symbols, Equations and Definitions in Pharmacokinetics (American College of Clinical Pharmacology 1982). Coded compounds with the prefix SN refer to the register of the National Survey of Antimalarial Drugs (USA), and those with the prefix WR to the register of the Walter Reed Army Institute of Research, Washington, DC.

MECHANISM OF ACTION OF ANTIMALARIAL DRUGS

The mechanism of action of antimalarial drugs has been reviewed by Peters (1974), Elslager (1974), Peters & Howells (1978) and Warhurst (1980). This section, therefore, will only attempt to bring the reader up-to-date with current views.

FOLATE PATHWAY ANTAGONISTS

Sulfonamides and sulfones

Sulfonamides and sulfones act, in principle, on all multiplying stages in the life-cycle of the malaria parasites. Activity has been demonstrated in animal models on both tissue and blood schizogony as well as on sporogony in the insect vector. However, activity against human parasites appears to be restricted to an effect on the asexual blood cycle. No effect is observed on the gametocyte stages of any species.

The antiplasmodial action of sulfonamides was reported as early as 1937 and many derivatives of these compounds were used with varying success against human malaria. However, their slow and short-lasting action and the need for high and potentially toxic doses resulted in their use being

discontinued. Interest in these compounds revived with the development of long-acting compounds such as sulfadoxine and sulfadiazine and with the development and spread of resistance to 4-aminoquinolines. The sulfonamides are not used alone but usually in combination with dihydrofolate reductase inhibitors such as pyrimethamine with which they are synergistic.

The selective toxicity of the sulfonamides and sulfones resides in the fact that they compete with para-aminobenzoic acid (PABA) for binding sites to the enzyme, dihydropteroate synthase (EC 2.5.1.15) which catalyses the condensation of PABA with phosphorylated pteridine to form dihydropteroate. This in turn is converted to dihydrofolate which is used as a cofactor in the formation of precursors of purines required for nucleic acid synthesis (Fig. 51.1). Mammalian cells can produce their dihydrofolate directly from dietary folic acid.

Dihydropteroate synthase has been isolated from *P. berghei* and *P. chabaudi* and has a K_m for PABA of 0.21–0.28 μmol/l, and K_i values for dapsone (DDS), sulfadiazine, sulfathiazole and sulfanilamide have been shown to be comparable to those determined from bacterial sources (Ferone 1977).

Thus, it is classically thought that the malaria parasites utilize PABA as a precursor for the formation of tetrahydrofolate whereas the host utilizes folic acid. This may, however, be an oversimplification since McCormick & Canfield (1972) demonstrated that folic acid did, in fact, interfere with the activity of sulfalene against *P. knowlesi* in vitro and this has recently been confirmed with studies on the activity of a number of sulfonamides including sulfadoxine against *P. falciparum* in vitro (Milhous 1983; Watkins et al 1985). These results could be explained by the fact that dihydropteroate synthase from *P. berghei* can utilize para-aminobenzoylglutamate (PABG) as an alternative substrate to form dihydrofolate, although the apparent K_m for PABG is about 100 times higher than that for PABA (Ferone 1977). The PABG which is an alternative substrate for dihydropteroate synthase in *P. berghei* (Blakely 1969) could arise from the cleavage of folates. Such a mechanism has already been proposed by Rollo (1955) to explain the competitive reversal of sulfonamide inhibition by folic acid in *P. berghei* instead of non-competitive reversal which might have been expected if folic acid had been used intact. However, folic acid was markedly more potent than either PABA or PABG as an antagonist of sulfadoxine, which suggests a mechanism involving the intact folic acid molecule

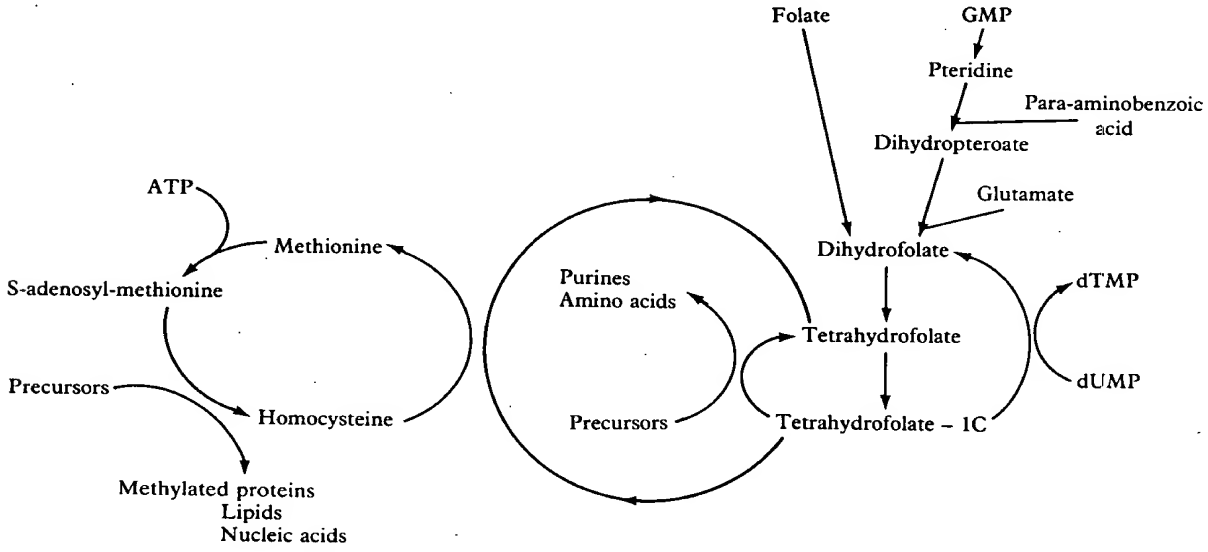


Fig. 51.1 Folate metabolism (GMP = guanosine monophosphate; dTMP = d–thymidine monophosphate; dUMP = d–uridine monophosphate)

(Watkins et al 1985). Alternatively, folic acid may act in some other way, e.g. by inhibiting sulfadoxine transport into the cell. Further studies are required to elucidate the true mechanism, but the above results do suggest that *P. falciparum* can utilize some of the folate within the human erythrocyte.

Dihydrofolate reductase inhibitors

Although the selective toxicity of sulfonamides and sulfones depends on the possession by the malaria parasite of a metabolic pathway distinct from that of the host, both the parasite and the host convert dihydrofolate to tetrahydrofolate using dihydrofolate reductase (EC 1.5.1.3). The selective action of 2,4-diaminopyrimidines such as pyrimethamine and the triazines such as cycloguanil resides in the greater affinity of the drug to the parasite enzyme than to the host enzyme.

Dihydrofolate reductase is a key enzyme in folate metabolism since it converts the pteridine ring to the tetrahydro reduction state required for reactions in which the folate cofactors are synthesized. It was the first enzyme of the folate pathway to be demonstrated in malaria parasites (Ferone et al 1969) and has since been reported in many species to have a molecular weight five to ten times higher than that found in bacteria, birds and mammals (Ferone 1977). Pyrimethamine binds 10^2–10^3 times more tightly to the plasmodial enzyme than to that of the host and similar values have been observed for trimethoprim, cycloguanil and several dihydrotriazines. The concentrations of pyrimethamine and trimethoprim required to inhibit the isolated enzyme of *P. knowlesi* are very close to those which are inhibitory to the growth of the parasite in vitro, e.g. the concentration for 50% inhibition was 1×10^{-9} M and 3×10^{-8} M for pyrimethamine and trimethoprim respectively (Gutteridge & Trigg 1971; McCormick et al 1971), these figures correlating with the reduced activity against human malaria of trimethoprim compared with pyrimethamine (Bruce-Chwatt et al 1981). From these studies and more extensive ones conducted by industry, it can be crudely predicted that compounds which do not selectively inhibit the plasmodial enzyme in a cell-free system would not be effective in vivo. Such an approach has led to the identification of several analogues of trimethoprim with superior activity against *P. berghei* in vivo.

Although it is clear that drugs such as the 2,4-diaminopyrimidines inhibit the dihydrofolate reductase of the parasite, there has been some controversy regarding the final result of this inhibition on the metabolism of the parasite. Of the various stages in the intraerythrocytic cycle, only the schizont stage is morphologically affected by pyrimethamine and trimethoprim, the drugs acting most markedly during chromatin division (McGregor & Smith 1952; Aikawa & Beaudoin 1968; Gutteridge & Trigg 1971). These observations correlate with the fact that the specific activity of dihydrofolate reductase increases most noticeably at or before the early schizont stage (Walter & Königk 1971).

Inhibition of dihydrofolate reductase results in a reduction in the pool of tetrahydrofolate cofactors which are used in most cells for the de novo synthesis of purines, methionine and thymidylate and for the interconversion of glycine with serine. Malaria parasites do not synthesize purine de novo (Sherman 1979), nor does unequivocal evidence exist for the synthesis of N^5-methyltetrahydrofolate and its utilization for methionine synthesis (Ferone 1977). So far serine is the only identified source of methyl groups of methionine and thymidylate synthesis in malaria parasites. Both *P. lophurae* (Platzer 1972) and *P. berghei* (Ferone 1984) contain serine hydromethyltransferase which converts serine to glycine with the formation of N^5, N^{10}-methylenetetrahydrofolate. This folate cofactor is utilized for the synthesis of thymidylate in a reaction catalysed by thymidylate synthase, an enzyme found in *P. lophurae* (Walsh & Sherman 1968), *P. chabaudi* (Walter et al 1970) and *P. berghei* (Reid & Friedkin 1973). The folate product in this reaction is dihydrofolate which must be reduced by dihydrofolate reductase to re-enter the tetrahydrofolate cofactor pool. Evidence exists which suggests that tetrahydrofolate dehydrogenase and thymidylate synthase exist as bifunctional proteins in *P. berghei* (Ferone 1984) and in *P. chabaudi* (Pattinakitsakul 1982).

Thus, the presence of enzymes of the thymidylate synthase cycle and the lack of utilization

of pyrimidines by the malaria parasite (Sherman 1979) indicate that the high affinity of parasite dihydrofolate reductase to pyrimethamine should result in inhibition of DNA synthesis by this drug. This is supported by the observation that the morphological effects of dihydrofolate reductase inhibitors are manifested at the schizont stage and that the major part of DNA synthesis occurs either during or shortly before schizont formation (Conklin et al 1973; Newbold et al 1982; Inselburg & Banyal 1984). In addition, thymidylate synthesis is inhibited by pyrimethamine in *P. knowlesi* (Smith et al 1977). However, Gutteridge & Trigg (1971, 1972) suggested that in addition to thymidylate synthesis other functions requiring folate cofactors might be inhibited. Apart from the 2,4-diaminopyrimidines, the quinazolines and triazines also exert their antimalarial activity by the inhibition of dihydrofolate reductase from the parasite. However, a derivative of the dihydrotriazine clociguanil, WR 99 210 (4,6-diamino-1,2-dihydro-2,2-dimethyl-1-[(2,4,5-trichlorophenoxy) propyloxy]-1,3,5-triazine hydrobromide) retained its activity against parasites resistant to pyrimethamine and cycloguanil (Peters et al 1975; Knight et al 1982). Whether this phenomenon can be explained purely on a genetic basis or whether it implies that WR 99 210 has another mode of action in addition to its role as an antifolate drug is worth investigating.

CHLOROQUINE AND RELATED BLOOD SCHIZONTOCIDES

Blood schizontocides such as chloroquine, quinine, mefloquine and amodiaquine, which only exhibit effects on the erythrocytic stages of malaria parasites, can be divided into two groups based on their chemical structure and their effects on the morphology of the parasite. In the first group, exemplified by chloroquine, there are two highly electronegative (easily protonatable) nitrogen atoms present and the first morphological effect is a swelling and fusion of the adjacent digestive vacuoles, followed by their sequestration in an autophagic vacuole, i.e. pigment clumping (Warhurst & Hockley 1967; Macomber & Sprinz 1967). In the second group exemplified by quinine, pigment clumping does not occur but the digestive vesicles may swell and the pigment within them becomes less electron-dense (Peters et al 1977a). Compounds in this group have only one highly electronegative nitrogen atom. They will competitively inhibit pigment clumping produced by compounds in the first group, indicating that the same receptor site is involved (Warhurst & Thomas 1975). Drugs of both groups are markedly concentrated by the intraerythrocytic parasite. This phenomenon was first observed in the late 1930s but more refined studies on *P. knowlesi* (Polet & Barr 1969), chloroquine-sensitive *P. berghei* and *P. falciparum* (Macomber et al 1966; Fitch 1969, 1970) later showed that the chloroquine concentration in parasitized erythrocytes was from 100- to 600-fold that observed in uninfected erythrocytes.

These observations led Fitch and his co-workers to investigate further the uptake of chloroquine and pharmacologically related compounds in rodent malaria parasites. Their data showed that chloroquine uptake was attributable to two processes: one was saturable with a high affinity and specificity for chloroquine, whereas the other was not saturable and of low affinity to the drug (Fitch 1969). The high affinity sites were absent in normal uninfected erythrocytes and significantly reduced in erythrocytes containing chloroquine-resistant parasites. Subsequently, it was shown that a similar drug-concentrating mechanism was present in erythrocytes infected with the relatively chloroquine-sensitive Camp strain of *P. falciparum* (Fitch 1970). Kramer & Matusik (1971) later proposed that the high affinity binding sites were associated with the membranes of 'free' *P. berghei* parasites whilst the low affinity sites were cytoplasmic.

Further experiments showed that chloroquine uptake in erythrocytes infected by both *P. berghei* and drug-sensitive *P. falciparum* required a supply of glucose and was inhibited by metabolic inhibitors, i.e. it was energy dependent (Fitch et al 1974a, b). Greater concentrations of glucose were required to stimulate uptake into erythrocytes infected with chloroquine-resistant *P. berghei* but drug accumulation by chloroquine-resistant *P. falciparum* was insensitive to glucose (Fitch et al 1975). Accumulation of other blood

schizontocides such as amodiaquine, quinacrine, quinine and mefloquine appears to occur by similar but not totally identical processes. For example, although the K_i's for amodiaquine and chloroquine accumulation were similar, uptake of amodiaquine was less dependent on glucose in a chloroquine-sensitive strain of *P. berghei* than that of chloroquine although glucose still stimulated amodiaquine uptake in a chloroquine-resistant strain (Fitch et al 1974b). In contrast, mefloquine uptake was neither stimulated by glucose nor inhibited by metabolic inhibitors. However, the uptake of both mefloquine and amodiaquine competitively inhibited that of chloroquine. Thus, it appears that those drugs which in general show cross-resistance in highly chloroquine-resistant strains of *P. berghei* are accumulated at the same site as chloroquine (Fitch 1972; Fitch et al 1974b, 1978).

Surprisingly, Fitch et al (1974c) demonstrated that mature erythrocytes treated with a non-specific protease also became able to accumulate chloroquine to a high degree. This led these workers to postulate that the sites which bind chloroquine at high affinity are made available in the infected cell by digestive processes of the parasite. As such processes require energy, this could account for the glucose requirement for drug uptake. It could also explain the anomalous behaviour of amodiaquine which Fitch and his colleagues suggest may be more readily accessible to a partially masked site.

Fitch and his collaborators have now extended their hypothesis to suggest that the high affinity binding site for chloroquine is ferriprotoporphyrin IX (FP) and that the selective antimalarial action of chloroquine in *P. berghei* is due to the complexing of the drug with this degradation product of haemoglobin. The basis of this hypothesis is as follows: erythrocytes infected with chloroquine-susceptible *P. berghei* produce FP (Fulton & Rimington 1953), some of which is available transiently (Fitch & Chevli 1981) to bind chloroquine with high affinity (Chou et al 1980; Chou & Fitch 1981). A chloroquine-FP complex then accumulates in infected cells resulting in damage to the cellular membranes including those of the parasite (Chou & Fitch 1981; Orjih et al 1981). A similar mode of action has been postulated for the action of chloroquine against *P. falciparum* (Fitch et al 1982). This hypothesis has been extended further subsequent to studies on the effect of chloroquine on phospholipid monolayers, suggesting that chloroquine has a dual effect. Firstly, chloroquine adsorbs onto the membrane interface thereby reducing the membrane's negative surface potential and allowing the more negatively charged FP to intercalate into the membrane. Secondly, it is suggested that FP and chloroquine form a complex which in turn causes lysis as postulated by Fitch (Ginsburg & Demel 1983, 1984).

This is an attractive hypothesis since the structure of FP corresponds with the hypothetical receptor predicted from structure/activity relationships (Fitch et al 1974b; Warhurst & Thomas 1975). In addition the K_d of chloroquine with malaria-infected erythrocytes correlates well with the K_d of the binding of chloroquine to the FP dimer. However, there are still inconsistencies which require explanation. For example, it is not clear how a transiently available receptor such as FP is capable of binding large amounts of drug which are concentrated in the digestive vacuoles. It is also difficult to explain the patterns of resistance to various blood schizontocides on the basis of this hypothesis, although the mode of action of a drug and the mechanism of resistance to it may not be directly related (see below).

Another problem with Fitch's hypothesis is that, as FP is more toxic than the chloroquine-FP complex in vitro (Orjih et al 1981), it may be similarly so in vivo. However, Banyal & Fitch (1982), using lysates of *P. berghei*, suggest that the parasite contains FP binding substances and that the mode of action of chloroquine may be to shunt FP away from a non-toxic complex with those substances and into a toxic chloroquine-FP complex. Evidence for the presence of such substance, i.e. protein(s) synthesized by the parasite, has been provided by Ashong & Warhurst (1985).

Antimalarials of the arylamino alcohol type including quinine, quinidine, cinchonine, cinchonidine, mefloquine and the phenanthrene-methanols also form complexes with FP. It is noteworthy that the inactive epimer of quinine, 9-epiquinine, does not.

Much attention has also focused on the hypothesis that chloroquine functions as a lysoso-

trations achieved with both chloroquine and these drugs indicate that their reaction with DNA must have some significance.

Evidence for the interaction of chloroquine with parasite cell membranes has been produced by Wunderlich et al (1981) who showed that, two hours after treatment with the drug, the rough endoplasmic reticulum of *P. chabaudi* disappears with a concomitant appearance of local agglomerations of smooth tubular membranes and the apposition of the nuclear envelope by smooth cisterna. In this context, Königk & Putfarken (1985) reported that ornithine decarboxylase (EC 4.1.1.17) which is involved in polyamine synthesis required for the stabilization and fusion of membranes and for protein synthesis is inhibited by chloroquine. Ornithine decarboxylase activity is maximal at the late trophozoite stage of *P. falciparum*, a stage which is most sensitive to chloroquine (Yayon et al 1983). In addition, Warhurst & Williamson (1970) reported that ribosomal RNA was degraded in *P. knowlesi* parasites treated with chloroquine. This degradation occurred after but not before autophagic vacuole formation. Chloroquine, however, did not affect the parasite, parasitophorous vacuolar or host cell membranes as judged by freeze fracture techniques. Wunderlich et al (1981) therefore suggest that their electron-microscope findings correlate with the biochemical studies of Warhurst & Williamson and with the fact that chloroquine does not induce major effects on the activities of some enzymes associated with the host cell plasma membrane, but does inhibit ornithine decarboxylase (EC 4.1.1.17) from the parasite (Königk et al 1981). This enzyme is the first step in the synthesis of polyamines necessary for the stabilization of ribosomes and cell membranes.

In addition, chloroquine and also quinine may inhibit invasion of the host cell by the parasite. Although Langreth et al (1978) failed to detect an effect on this process with concentrations of 2μg/ml of chloroquine, Hommel et al (1979) showed that pretreatment of the host cell with higher concentrations (2.5 μg/ml) reduced the susceptibility of monkey cells to *P. knowlesi* even though the residual concentration in the culture medium did not exceed 0.4 μg/ml. These latter authors suggest that this might explain why a serum level of 0.1 μg/ml or less (achievable by standard prophylactic doses) is effective whilst showing no effect on protein synthesis in vitro (Richards & Williams 1973).

ANTIBIOTICS

The first reports of an antimalarial effect of antibiotics appeared in 1949 when chlortetracycline was found effective against avian and human malaria parasites (Coatney et al 1949b; Cooper et al 1949). Coatney & Greenberg (1952) further reported that only nine of 31 antibiotics tested showed any antimalarial activity and of these only chlortetracycline, oxytetracycline and chloramphenicol were sufficiently well tolerated and active to be of potential value to malaria chemotherapy. All three compounds showed causal prophylactic as well as blood schizontocidal activity. Subsequent studies however showed that the effects of these drugs were slow to be manifested in comparison to drugs such as chloroquine, with the result that they were at that time considered to be of limited practical value.

The subsequent development and spread of drug-resistant falciparum malaria has prompted a reconsideration of the value of antibiotics as potentially useful antimalarials and their use, particularly of tetracycline, in combination with quinine for the radical cure of multidrug-resistant infections. Other antibiotics such as clindamycin, lincomycin, minocycline, doxycycline and erythromycin have been shown to have blood schizontocidal effects and minocycline and doxycycline have useful activity against the liver stages.

Erythromycin has been shown to have a synergistic effect with chloroquine against chloroquine-resistant *P. berghei* (Warhurst et al 1976). Warhurst (1977) subsequently suggested that the raised concentration of chloroquine inside the parasite would increase the permeability of the mitochondrial membrane to erythromycin, thus resulting in an effect on mitochondrial protein synthesis. He explained the lack of synergism between the two drugs in chloroquine-sensitive parasites by the masking of the erythromycin

malarials 1982a). More recent studies suggest that the complete molecule may be necessary for full activity (Warhurst, personal communication). Dihydroartemisinin, the reduction product of Qinghaosu and probably a major metabolite, retains the peroxide bridge and is more potent than the parent molecule (Gu et al 1981; Chawira et al 1985). Electron-microscope studies have also been performed on the effect of these drugs on *P. berghei, P. knowlesi, P. inui* and *P. falciparum*. These indicate that the first observable change produced by Qinghaosu in *P. berghei, P. knowlesi*, and *P. falciparum* was damage to the parasite membranes. Such damage may include swelling and spiral deformation of the food vacuole membranes as well as swelling of the parasite limiting membrane, the mitochondrial membrane, the nuclear membrane, the endoplasmic reticulum, and finally the formation of autophagic vacuoles (China Co-operative Research Group on Qinghaosu and its Derivatives as Antimalarials 1982c; Ellis et al 1981, 1985). Although similar changes occurred in *P. inui*, they were observed several hours after the swelling of the parasite mitochondria suggesting that, at least in this species, the alterations in membranous structures are secondary to mitochondrial damage (Jiang et al 1985). This apparent discrepancy may relate to differences in the mitochondrial morphology in the various species.

It has been suggested that the mode of action of Qinghaosu and its derivatives may be on parasite protein synthesis (Gu et al 1983) since inhibition of incorporation of ^{3}H-isoleucine into proteins of *P. falciparum* in vitro was observed within one hour of drug administration. The relatively slow onset of inhibition of ^{3}H-hypoxanthine incorporation into parasite nucleic acids when compared with the rapidity of action of these drugs in vivo suggests that nucleic acid synthesis is not the primary target of the drug (Z L Li et al 1983). Qinghaosu apparently does not inhibit carbohydrate metabolism. Although these results strongly suggest an initial action of these drugs on protein synthesis, inhibition of uptake of precursors into macromolecules does not necessarily indicate a primary mode of action on the macromolecules themselves. Thus, it is important to study the effect of these drugs on both cell-free protein synthesis and transport systems. Preliminary results already indicate that the parasite infected cells concentrate drugs of this series in a similar but not identical manner to that observed with 4-aminoquinolines (Gu et al 1984). Qinghaosu has been reported to affect polyamine metabolism of *P. falciparum* in vitro so that putrescine levels were depressed whereas spermine and spermidine levels were elevated. Artesunate, the hemisuccinyl derivative of Qinghaosu, does not inhibit the digestion of haemoglobin (quoted by Klayman 1985).

The discovery of the antimalarial effect of Qinghaosu has stimulated the search for similar antimalarial plant products. There are numerous naturally occurring terpenes which, like Qinghaosu, possess peroxide moieties. Ascaridole and several hydroperoxide terpenes have been tested by the Walter Reed Army Institute of Research but none has shown potential for development of antimalarial drugs (Klayman 1985). In contrast, Yingzhaosu isolated from *Artabotrys uncinatus* possessed antimalarial activity (Song Zhen-Yu, personal communication) and a peroxide bridge (Liang et al 1979a, b).

In vitro testing with *P. falciparum* has also shown that certain quassinoids from the plant family Simaroubaceae possess significant antimalarial activity (Trager & Polonsky 1981; Guru et al 1983). The most active compounds with IC_{50} values in the range of 1–5 ng/ml were bruceantin, bruceantinol, brusatol, isobruceine A, glaucarubinone and simalikalactone D. Ailanthinone, glaucarubin, holacanthone, samaderine E, 6-α-senecioyloxychaparrinone and undutatone had IC_{50} values of 6–65 ng/ml, whereas chaparrin and glaucarubol showed relatively low activity (Bray et al 1985a). These findings indicate that there is some correlation between antimalarial activity and the presence of an ester group at either C-6 or C-15 and that the oxygen bridge of the pentacyclic structure at C-20 may be either to C-11 or to C-13.

Antimalarial activity in vitro has also been demonstrated by the limonoids, gedunin, dihydrogedunin, nimbolide and nimbinin. Nimbolide has been shown to have activity against chloroquine-sensitive *P. berghei* in vivo (Bray et al 1985b).

fective against the persistent tissue stages of *P. vivax* or *P. ovale*. This drug acts on all stages of the malaria life-cycle, but is generally only used as a tissue schizontocide and a gametocytocide because of its significant toxicity in man and its limited blood schizontocidal activity.

Primaquine as well as the naphthoquinone, menoctone, and the 8-aminoquinoline, WR 225 448, have been shown to cause mitochondrial swelling in both the exoerythrocytic and erythrocytic stages (Beaudoin & Aikawa 1968; Aikawa & Beaudoin 1969; Howells et al 1970; Peters & Robinson 1984b; Peters et al 1984a). Moreover, ^{3}H-primaquine or a metabolite was detected within the mitochondria of tissue stages of *P. fallax* at the same time as mitochondrial swelling occurred (Aikawa & Beaudoin 1969). This has led to the hypothesis that such tissue schizontocides act by inhibiting mitochondrial respiration in the parasite. It is not known whether this occurs by a mechanism similar to that suggested for naphthoquinones. Primaquine has also been shown to inhibit the binding and entry of *P. berghei* sporozoites into a human hepatoma cell line in vitro (Schwartz & Hollingdale 1985).

The molecular mechanism of the mode of action of primaquine is unknown although it is generally accepted that its metabolism in the mammalian host has a role to play in both its antimalarial and toxic effects. Isomerism of the molecule may also play a role in these effects since it has been shown that the positive isomer of primaquine is at least four times more toxic to mice than the negative form of the drug whereas the opposite relationship exists in the toxicity of the two forms to rhesus monkeys (Carson 1984). Clearly more work has to be carried out on the mode of action and toxicity of primaquine and other potential tissue schizontocides. This has become possible by the development of new sensitive, specific and relatively inexpensive assay systems for these compounds and their metabolites and of in vitro systems in which the efficacy and toxicity of the metabolites can be tested.

Preliminary tests have shown that primaquine, demethylprimaquine, 5-hydroxyprimaquine and 5-hydroxy-6-demethylprimaquine have gametocytocidal activity in vitro against *P. falciparum* (Bhasin & Trager 1987) whereas only the parent drug has shown in vivo activity in a rodent model (Peters & Robinson 1987). In vitro and in vivo tissue schizontocidal activity has been only observed with primaquine (Hollingdale 1987; Peters & Robinson 1987). Truly causal prophylactic activity has only been observed in a rodent model with the parent drug and 5-hydroxyprimaquine (Peters & Robinson 1987). Surprisingly causal prophylactic and gametocytocidal activity has also been observed with certain new 8-aminoquinolines lacking the 8-N side chain (Peters & Robinson 1987). Further studies are clearly required in this area. The methodology now exists for the identification of primaquine metabolites and the determination of their role in the efficacy and toxicity of the parent compound.

MECHANISMS OF DRUG RESISTANCE

Drug resistance in microorganisms may be due to a variety of mechanisms including non-genetical physiological adaptations of the organisms to the drug, genetical changes such as mutational events followed by drug selection, non-adaptive changes or a combination of all these mechanisms. A remarkable feature of malaria parasites is the apparent frequency with which drug-resistant organisms occur in nature and the relative ease with which resistant lines of *Plasmodium* can be developed in the laboratory to any antimalarial compound. A wide variety of techniques have been used experimentally to produce drug-resistant parasites (Peters 1984). However, considerable controversy still surrounds the question of whether a resistant line is more likely to be produced by slowly increasing the drug pressure or by the administration of a single high dose of the drug. In reality, there can be little doubt that different mechanisms account for different types and levels of resistance for any given compound (Padua 1981).

In the past, the majority of studies on drug resistance have been made using avian and rodent models. However, the availability of suitable techniques for cultivating *P. falciparum* in vitro and for studying the molecular biological aspects of this parasite should now lead to studies in the

ations in dihydrofolate reductase from resistant parasites (Sirawaraporn & Yuthavong 1984). They have observed increases in the K_i for pyrimethamine, the K_m for the substrate and the number of catalytic sites for the enzyme in resistant parasites. There was also a reduction in the turnover of the enzyme but no increase in the overall specific activity in resistant parasites. In contrast, Kan & Siddiqui (1979) failed to find qualitative differences between the dihydrofolate reductase extracted from pyrimethamine-sensitive and -resistant lines of *P. falciparum*. Instead, they reported that the specific activity of the enzyme was 30–80-fold higher in the resistant (Uganda Palo Alto) than in the sensitive line (Vietnam Oak Knoll). Thus, it is not clear at this stage whether gene amplification occurs in malaria parasites resistant to pyrimethamine, although electron-microscopical studies by Lanners & Trager (1984) indicate the presence in a resistant line of *P. falciparum* of intranuclear structures which the authors suggest may be related to gene amplification.

It does not appear that resistance to pyrimethamine, in contrast to chloroquine (see below), is a dominant character offering a selective advantage. Rosario et al (1978) performed a growth competition experiment with *P. chabaudi* starting with a population of 50% resistant and 50% sensitive parasites. After 30 days in the blood of mice not exposed to the drug, 56 sensitive and 11 resistant clones were isolated. It was therefore concluded that pyrimethamine resistance is neutral or possibly selectively disadvantageous.

RESISTANCE TO SULFONAMIDES AND SULFONES

Lines of *Plasmodium* resistant to sulfonamides or sulfones may be developed in the laboratory either by exposing the parasites to single heavy or repeated subcurative doses of the drugs or by withholding the cofactor para-aminobenzoic acid (PABA). In either case, it appears that the parasites must survive by by-passing the step at which PABA is incorporated into dihydropteroate. It is possible that they may be able to do this by utilizing host cell folates (see p. 1582). Lines of *P. berghei* resistant to these compounds develop less rapidly in mice than the parent line since resistant organisms are obligate parasites of reticulocytes. Resistance to these compounds is, however, a stable character and is carried through all stages of the life-cycle when the parasite is cyclically transmitted (Peters 1971).

Resistance to sulfonamides and sulfones is usually associated with hyposensitivity to dihydrofolate reductase inhibitors and the synergistic action of these two types of compounds is sufficiently marked to overcome even a high level of resistance to one or the other (Peters 1971). Surprisingly little is known on the biochemical basis of resistance to these compounds.

RESISTANCE TO 4-AMINOQUINOLINES

In contrast to that of pyrimethamine, resistance to chloroquine in human malaria species is restricted to *P. falciparum*. All other human species remain so far fully susceptible to this and other 4-aminoquinolines. Also, chloroquine resistance is less easily obtained in laboratory animal models, although this is complicated by the fact that rodent species of *Plasmodium* vary greatly in their response to the drug. Single step resistance is difficult to obtain, selection of resistant forms normally requiring prolonged treatment with gradual increases in the drug dose. In some instances resistance is unstable, suggesting that either non-genetic or extrachromosomal mechanisms may be involved.

Genetic studies on chloroquine resistance have so far been carried out only with *P. chabaudi*. Rosario (1976) obtained a parasite line which was resistant to a low level of the drug after a long series of drug treatments. The resistance was stable and, following a cross with a chloroquine-sensitive line, the resistance character underwent recombination with other markers including pyrimethamine resistance, indicating a normal dominant Mendelian inheritance.

In subsequent studies, Padua (1981) exposed this same line to even higher progressive doses until a highly resistant line was obtained. When this line was crossed with a sensitive one, clones exhibiting various levels of resistance, ranging from complete sensitivity to high resistance, were

of *P. yoelii* are not obligatory parasites of these cells and cyclically transmitted resistant lines of *P. berghei* develop in both reticulocytes and mature red cells (Peters et al 1970; Schuurkamp 1982).

Red cells infected with chloroquine-resistant *P. berghei* and *P. falciparum* accumulate less chloroquine than those infected with comparable chloroquine-susceptible strains (Macomber et al 1966; Fitch 1969, 1970). This led Fitch and his colleagues to extend their hypothesis on the mode of action of this drug (see p. 1575) and to suggest that chloroquine resistance in *P. berghei* resulted in a lack of production of ferriprotoporphyrin IX in red cells infected with resistant parasites. This is an attractive hypothesis for which a great deal of evidence has been accumulated in the model system, but how far it can be extrapolated to other isolates of *P. berghei* and to *P. falciparum* is not clear. Resistant lines of *P. berghei*, other than those used by Fitch and his co-workers, produce normal malaria pigment and more recent studies using non-invasive techniques indicate that haemoglobin digestion occurs even in the highly resistant lines of *P. berghei*. In addition, ferriprotoporphyrin IX has not been detected in *P. berghei* parasites (Yayon et al 1984b). Haemoglobin digestion in *P. falciparum* differs from that in *P. berghei* and malaria pigment is found in all isolates of this species including those which are highly chloroquine-resistant. Fitch et al (1982) explain these apparent anomalies by suggesting that ferriprotoporphyrin IX is more efficiently sequestered in resistant parasites so that it is not available for chloroquine binding. Evidence for this is inconclusive at this stage. Particularly, it has to be shown that ferriprotoporphyrin actually exists, even transiently, as a free molecule and that the mechanisms which have been suggested from studies in model systems occur under physiological conditions in malaria parasites.

RESISTANCE TO QUININE AND MEFLOQUINE

Several reports have indicated that a diminished response to quinine could be obtained when isolates of *P. falciparum* are passaged under drug pressure in man (McNamara et al 1967) or in *Aotus* (Glew et al 1978). This has now been shown to have important operational significance since comparative in vivo and in vitro data from Thai children confirm a diminished sensitivity of *P. falciparum* in Thailand to this drug (Chongsuphajaisiddhi et al 1981). As quinine and a new quinolinemethanol drug, mefloquine, are similar both in their structure and mode of action, quinine resistance may be a potential threat to the use of mefloquine. Recently, Suebsaeng et al (1986) have shown a parallel reduction in the sensitivity of *P. falciparum* to both drugs in Thailand during the period 1982–84.

Mefloquine resistance has been induced in rodent malaria parasites (Peters et al 1977b; Merkli et al 1980) and disturbingly it appears to arise more quickly from a chloroquine-resistant than a chloroquine-sensitive strain (Peters et al 1977b). Decreased susceptibility of *P. falciparum* to mefloquine has also been reported following maintenance of the parasite under drug pressure in continuous culture in vitro (Brockelman et al 1981). Resistance in all these isolates was unstable, reverting to sensitivity in the absence of drug pressure. The mefloquine-resistant lines remained sensitive to chloroquine, but were cross-resistant to quinine and to a synthetic quinine analogue. However, stable resistance to mefloquine in vitro has been obtained in clones of *P. falciparum* (Desjardins, personal communication).

Although results obtained in rodent models might not be applicable to the human situation, all steps should nevertheless be taken to protect mefloquine once it comes into operational use. Recent work has concentrated on the development of drug mixtures of mefloquine since Peters (1974) had shown that the use of such mixtures can, in certain circumstances, inhibit the development of drug resistance to the individual components even though it does not completely prevent the process. Merkli et al (1980) have shown that this applies also to a combination of mefloquine with sulfadoxine and pyrimethamine. In this study, a line resistant to the triple combination was produced which remained sensitive to chloroquine but developed a modest resistance to sulfadoxine and a solid resistance to pyrimethamine when this was used alone. In view of the known modes of

gametocytocidal action of primaquine. Although infections with some strains of *P. vivax* need to be treated with high dosages of this drug to effect radical cure, true drug resistance in these stages is not a problem. Primaquine also has limited blood schizontocidal activity and many studies have been carried out on the development of primaquine resistance by these stages of rodent malaria parasites. Such resistance develops rapidly when *P. berghei* is exposed to a slowly increasing drug pressure in vivo (Merkli & Peters 1976). Peters (1966) noted that parasitaemia developed more slowly in mice infected with a primaquine-resistant line of *P. berghei* than with the drug-sensitive parent line. Resistance to primaquine in the line developed by Peters was unstable in the absence of drug pressure, whereas Bishop (1967) found that resistance to primaquine in *P. gallinaceum* was more stable than that observed in *P. berghei*.

Primaquine-resistant parasites show morphological differences from sensitive ones. Howells et al (1970) reported that parasites which survived exposure to primaquine appeared to contain a greater number of mitochondria than did normal parasites. It seems possible therefore that one mechanism of resistance to this compound could be the generation of an increased number of these organelles to compensate for any drug effect. Similar mitochondrial changes have been noted following exposure of the blood stages of *P. berghei* to the naphthoquinone, menoctone. Another feature of primaquine-resistant *P. berghei* was the appearance of the haemozoin of the asexual blood stages. Electron-microscope studies indicated that pigment formation in these parasites appeared to take place by a process similar to that observed in avian malarias and *P. falciparum* rather than that observed in primaquine-sensitive *P. berghei*. The reasons for these changes and the biochemical basis of primaquine resistance in malaria parasites is unknown.

PHARMACOKINETICS

Quinine was introduced for the treatment of malaria long before the development of the science of the fate of drugs in the host, i.e. pharmacokinetics. The dose regimens of quinine, both for treatment and suppression, emanated from experience. This empirical approach yielded a surprisingly close approximation to the 'ideal' regimens established on the basis of modern pharmacokinetics, probably helped by the relatively short half-life of the drug and in spite of the proximity of effective and toxic doses. Conditions with regard to other antimalarial drugs may be vastly different, especially when they have a long and variable half-life. In view of the relatively recent development of sensitive assay techniques such as high performance liquid chromatography (HPLC), gas chromatography (GC), thin layer chromatography (TLC), spectrofluorometry (SF), and electron capture gas chromatography (ECGC), it is not surprising that detailed pharmacokinetic information on the standard 4-aminoquinolines (chloroquine and amodiaquine) became available only in the 1980s. More extensive data on pyrimethamine and sulfadoxine were recently generated in the course of the development of the combination with mefloquine. The latter compound may be considered as one of the best documented antimalarial drugs.

The elaboration of sensitive assay techniques is also crucial to the development of new drugs. Progress of many a promising antimalarial compound has been halted for the lack of adequate assay technologies with the result that these candidate drugs remain on the shelf, their potential not being further explored.

The advent of new and yet more sensitive assay techniques such as laser fluorometric detection interfaced with HPLC (Zare 1984) may not only overcome these problems but also extend the possibilities of pharmacokinetic investigation with regard to standard antimalarial drugs. Antimalarial drugs are different from other medicaments in as much as their target sites are the malaria parasites which are either in the blood or in the liver. Drugs capable of killing merozoites or blocking their interiorization have not yet been discovered. Thus blood schizontocides need to be available in an adequate concentration in the blood and to reach the parasite across the erythrocyte membrane. Tissue schizontocides need to reach the exoerythrocytic forms within the hepatocyte. Thus, principles applicable to the pharmacokinetics of antibiotics (Bergan 1981) may

method and from shortcomings in differentiating between chloroquine and its metabolites. A spectrofluorometric assay (McChesney et al 1967) brought some improvement, but major progress was made only after the introduction of a sensitive high performance liquid chromatography (HPLC) method for the determination of chloroquine and its metabolites by Bergqvist & Frisk-Holmberg (1980). Independently, a similar (though not identical) and highly sensitive HPLC technique was developed by Oosterhuis et al (1981) which is suitable for measuring chloroquine and its main metabolites desethylchloroquine and bidesethylchloroquine. Alván et al (1982) simplified the method of Bergqvist & Frisk-Holmberg (1980), while largely maintaining specificity and sensitivity for the detection of chloroquine and its desethyl metabolite. The method of Bergqvist & Frisk-Holmberg has a lower sensitivity limit of 0.5×10^{-9} M chloroquine following extraction from plasma or urine. At this level, the relative standard deviation was 11.4% and the recovery 100% for chloroquine; similar values were obtained with the desethyl metabolite. Plasma and urine samples with chloroquine concentrations higher than 0.2×10^{-6} M can be used directly in the assay without prior extraction and the recovery rate in plasma was higher than after extraction both for chloroquine and the desethyl metabolite. In contrast, chloroquine has to be extracted from whole blood before the HPLC method can be employed, yielding 87% recovery (with ± 2.5% standard deviation) at 10×10^{-9} M chloroquine and 89% recovery (with ± 1.1% standard deviation) at 10×10^{-9} M desethylchloroquine. Recovery was less (75% and 70% respectively) at 0.8×10^{-6} M chloroquine and desethylchloroquine, and the standard deviations were slightly increased, but still within narrow limits. Assays in urine, using the direct injection HPLC method, yielded high recovery rates (98–100% for chloroquine and 97–99% for desethylchloroquine) at concentrations between 0.5 and 50×10^{-6} M, with generally small (<± 2%) standard deviations.

With the HPLC method of Oosterhuis et al (1981) chloroquine may be detected down to a concentration of 3.1×10^{-9} M, and the recovery rates for chloroquine, desethylchloroquine and bidesethylchloroquine were 83.3%, 79.7% and 51.7% respectively over the range of 3.1×10^{-9} M to 1.25×10^{-6} M. The HPLC technique of Alván et al (1982) yielded an absolute recovery of chloroquine and desethylchloroquine of 88%, 80% and 88% from plasma, red blood cells and urine respectively. The detection levels for chloroquine and desethylchloroquine were 3.1×10^{-9} M and 1.7×10^{-9} M respectively. The precision was ± 3.5% at 0.16×10^{-6} M chloroquine and ± 5% at 0.087×10^{-6} M desethylchloroquine in plasma.

The corresponding precision values for assays from red blood cells were ± 4% for chloroquine and ± 17% for desethylchloroquine. As emphasized by Alván et al (1982), the avoidance of haemolysis during the processing of blood samples is most important since red blood cells have approximately five times the chloroquine concentration of plasma (Adelusi et al 1982; Gustafsson et al 1983). Thus haemolysis may result in the reading of unduly high plasma concentrations which could lead to an erroneous pharmacokinetic interpretation.

Chloroquine assays have become increasingly important as a complement to in vivo and in vitro tests of drug sensitivity. Suitable methods for immediate analysis in the field are not yet available, but the technique of Patchen et al (1983) permits a quantitative analysis of chloroquine and desethylchloroquine from filter paper absorbed fingerstick blood. It is based on elution, extraction and HPLC with fluorescence detection. The limit of detection is approximately 1.5×10^{-8} M for both chloroquine and its main metabolite, using 100 μl fingerstick samples. The assay precision for both chloroquine and desethylchloroquine compares favourably with that of other HPLC assays. Storage of the filter paper samples at room temperature over 12 weeks gave no indication of changed reactivity. Comparative analysis of chloroquine and desethylchloroquine in venous blood and fingerstick blood from the same individual produced very similar results. The advantages of blood sampling through fingerstick rather than venepuncture are obvious. The ease with which the specimens can be handled will render sampling in the field quite feasible.

Before the 1980s, there were only a few studies of the absorption and bioavailability of chloroquine; the interpretation of their results requires

erythrocytes reflects also the selective uptake of chloroquine by the parasite. Thus Adelusi et al (1982) found a ratio of 21:1 between erythrocyte and plasma chloroquine concentrations at the height of parasitaemia in children suffering from falciparum malaria. After the complete disappearance of the parasites, the ratio dropped to 5.3:1 and stabilized at this level. Gustafsson et al (1983) found a ratio of 4.8:1 in healthy adult males from Sweden after a single dose of 300 mg (base). In France, Verdier et al (1984) obtained a mean ratio of 8.5:1 in nine healthy adults (five females, four males) whose blood was taken 12 hours after having received the last of 10 daily doses of 100 mg chloroquine (base). Their studies showed that the chloroquine assayed in red blood cells and plasma together accounted only for 78% of chloroquine found in the whole blood. This indicates a high uptake of chloroquine by other cellular elements of the blood which corresponds approximately to the uptake in the tissues. There were important individual variations in the ratio between erythrocytic and plasma chloroquine concentrations (3.7:1 to 15.6:1), in the percentage of chloroquine bound to blood cells other than erythrocytes (up to 58%), and in the plasma concentrations which varied between 0.23 and 1.20×10^{-6} M (mean 0.76×10^{-6} M).

Plasma binding of chloroquine is not strong. Approximately 50–60% of the drug is bound to plasma proteins (Berliner et al 1948). Chloroquine binds less to human serum albumin than to plasma (Adelusi & Salako 1982b), suggesting that proteins other than albumin have a higher affinity to the drug.

In the human body chloroquine is metabolized by de-ethylation in the side chain to desethylchloroquine, the main metabolite, which has an antimalarial activity similar to that of the parent compound. Further de-ethylation leads to bidesethylchloroquine which has a strongly reduced antimalarial activity. Gustafsson et al (1983) observed peak plasma concentrations of desethylchloroquine in about 6 hours after the intravenous administration of 300 mg chloroquine (base). After oral administration of the same dose, the peak concentration of desethylchloroquine was generally reached earlier, at about the same time as that of the parent drug (mean 3 hours). After peak levels had been reached, the ratio of desethylchloroquine (DECq) to chloroquine (Cq) + DECq stabilized at a level between 0.20 and 0.30 (20–30%). The distribution kinetics of desethylchloroquine resemble apparently those of the parent drug. In a subject under chloroquine prophylaxis with weekly doses of 5 mg (base)/kg body weight, Churchill et al (1983) found much lower DECq ratios after the first dose as compared to the eighth week when a steady state had apparently been reached. During the first week the chloroquine whole blood concentrations descended from 1.07×10^{-6} M at 8 hours after drug intake to 0.19×10^{-6} M at 168 hours after drug intake. Those of the metabolite dropped from 0.20×10^{-6} M to 0.02×10^{-6} M over the same time span. The ratio of DECq : (Cq + DECq) (expressed as a percentage) which had shown a peak at 24 hours (18.2%) fell to 10% after 168 hours. The ratio was consistently higher and more stable after the administration of the eighth dose, with the lowest value of 37.4% at 8 hours after the dose and the peak of 46.1% at 72 hours after the eighth dose. During the eighth week the chloroquine whole blood concentrations descended from 1.97×10^{-6} M at 8 hours after drug intake, to 0.51×10^{-6} M some 168 hours after drug intake.

Verdier et al (1984) found rather large individual variations of the ratio of DECq:(Cq + DECq) in Madagassi patients who had received chloroquine orally at a total dose of 10 mg or 25 mg/kg body weight for the treatment of falciparum malaria. In whole blood specimens taken 9 days after the start of treatment the ratio, expressed as a percentage, varied between 27.6 and 44%, with a mean of 35.2%. While such variations may not play a major role with regard to the efficacy of the drug, where the absolute concentration will be important, they may have a bearing on side effects. Olatunde (1971) found higher chloroquine and lower desethylchloroquine concentrations in the skin of persons prone to itching after chloroquine administration as compared to other African subjects who did not suffer from drug-induced pruritus. The chloroquine plasma concentrations were similar in both groups.

Earlier estimates of the half-life of chloroquine

tration when a loading period of four weeks with 600 mg chloroquine (base) weekly preceded the weekly administration of 300 mg. With this regimen the peak concentrations did not reach the level associated with toxic manifestations.

Little is known about the pharmacokinetics of chloroquine in malnourished individuals. The studies of Krishnaswamy (1978, 1983) have produced evidence for significant changes of drug metabolism and pharmacokinetics in malnutrition. This applies, for instance, to rifampicin (Polasa et al 1984). It would be useful to conduct similar investigations also with chloroquine in view of its peculiar distribution in blood and tissue. This may have a bearing also on assessing the risk of toxic side effects and drug interactions. The latter have to be viewed in perspective with the marked ability of chloroquine to inhibit drug oxidation (Breckenridge et al 1984).

An interesting aspect of chloroquine is the relative activity of its two optical isomers. Haberkorn et al (1979) found the pure d-enantiomer to be significantly more effective against blood-induced *P. berghei* than the l-enantiomer or even the racemate. It is also significantly less toxic than both l-enantiomer and the racemate. Thus, chloroquine would not appear to be an example of a racemic compound with a toxicological and activity equivalence of its optical isomers as postulated by Steck (1972) and Korolkovas (1974). It is not known whether the difference between d- and l-chloroquine manifests itself also in a disparity of bioavailability and kinetics.

For the want of sensitive assay methods, the pharmacokinetics of amodiaquine remained for a long time quite obscure. From earlier investigations it was clear that little of the drug was eliminated in the form of the parent compound. It was only recently that four metabolites of amodiaquine were shown to be present in the blood of persons dosed with amodiaquine (Churchill et al 1985). The main metabolite has been identified as desethylamodiaquine, the second tentatively as 2-hydroxydesethylamodiaquine. Churchill et al (1985) developed a reverse-phase HPLC method that produces clear, reproducible, separate readings for amodiaquine and the two identified metabolites. Results obtained in two healthy adult volunteers dosed with amodiaquine (5 mg/kg per week) showed that practically no parent drug was detectable in the blood 4 hours after dosing, while desethylamodiaquine constituted the majority of processed drug, with a lesser though significant presence of 2-hydroxydesethylamodiaquine. These results suggest that amodiaquine is subject to an intense first pass phenomenon and that the biologically active compound is desethylamodiaquine.

In preliminary in vitro studies (Churchill et al 1985) this metabolite was slightly less active against *P. falciparum* than the parent drug. For routine in vitro sensitivity tests it clearly follows that desethylamodiaquine should be used in the future instead of amodiaquine. At this stage nothing is known about the biological activity of 2-hydroxydesethylamodiaquine since adequate quantities of this compound have only very recently become available.

Using the HPLC technique of Churchill et al (1985), Salako & Idowu (1985) studied amodiaquine and desethylamodiaquine concentrations in five healthy adult male Africans after the oral administration of 600 mg amodiaquine (base). Amodiaquine, the parent compound, was not detectable in erythrocytes, plasma or urine at any time after dosing. Desethylamodiaquine became detectable in the blood 45 minutes after dosing and reached peak levels in both plasma and red blood cells within 1½–2 hours. The peak was followed by a slow decline of the plasma concentration which, 24 hours after dosing, was approximately 30% of the peak concentration. Desethylamodiaquine remained detectable in the blood for 21 days. In the urine only desethylamodiaquine was detectable, the excretion during the first 24 hours accounting for 2.8% of the administered dose of amodiaquine. Contrary to chloroquine, no significant difference was seen between the desethylamodiaquine concentrations in plasma and red blood cells at any time of sampling between day 0 and day 21.

These results are only preliminary, but, with the reawakening interest in amodiaquine, an elucidation of the kinetics of this prodrug and its main metabolites may be expected now that a sensitive assay method has become available.

PRIMAQUINE

Little information on the pharmacokinetics of this 8-aminoquinoline was available until recently on account of the lack of appropriate assay methods

to quinine has recently decreased in areas affected by multiresistance, where the traditional standard treatment no longer provides reliable cure in a significant number of cases. Thus Chongsuphajaisiddhi et al (1979) observed that 4 out of 20 children receiving three times daily 8.3 mg quinine (base) per kg body weight for 14 days had recrudescences of falciparum malaria. These findings were indicative of inadequate curative quinine levels.

Chongsuphajaisiddhi et al (1981) conducted a study in 50 children suffering from falciparum malaria; 24 of the children received quinine 10 mg (base) per kg body weight every eight hours for 14 days, and 26 were given quinine adjusted to body surface based on an adult dose of 500 mg quinine (base) every 8 hours for 14 days. The drug was given by the oral route except in one severe case where the treatment was initiated with i.v. infusion. There was one case of resistance at the RI level and one at the RII level in the first group, and one RI case in the second. Quinine reached a peak level on day 2; the second group had consistently and significantly higher quinine blood levels as compared to the first group (peak 32.39×10^{-6} M against 25.35×10^{-6} M). In both groups, the quinine levels dropped off after day 2, apparently associated with the disappearance of fever, and reached relative stability as from day 6 until day 14, at a level below 20×10^{-6} M in the first group, and above 20×10^{-6} M in the second. Chongsuphajaisiddhi et al (1981) related the quinine levels to the minimum inhibitory concentrations (MIC) of quinine as assessed by the in vitro macromethod of Rieckmann et al (1968). The MICs for quinine varied between 8 and 26×10^{-6} M, with a mean of $14.88 \pm 4.51 \times 10^{-6}$ M. This indicates a very narrow margin between MIC and therapeutically obtained quinine concentrations. Specific investigations in children with treatment failures showed that the quinine serum levels were below the MIC for substantial periods; this was particularly striking in the case of an RII response, where the serum quinine concentration surpassed the MIC level for less than 48 hours only.

Confronted with a similar problem in the treatment of cerebral malaria, where the establishment of effective quinine levels will often decide the patient's fate, White et al (1983a) investigated the potential advantages of a quinine loading dose.

In a preliminary study, the quinine plasma concentrations were assessed in patients who had received standard treatment with i.v. infusions of quinine hydrochloride at a dosage of 10 mg/kg every 8 hours for six days, with an infusion time of four hours (White et al 1982). Later, 15 patients were treated with a single initial loading dose of 20 mg/kg quinine hydrochloride, good tolerability to such a dose having been demonstrated. This initial loading dose was followed by i.v. drips with 10 mg/kg every 8 hours until and including day 6. Another group of 12 patients received the standard treatment without a loading dose.

Clinically and parasitologically, the group with the loading dose became rousable earlier (32 ± 20.7 hours as opposed to 43 ± 21.5 hours), regained full consciousness earlier (46 ± 25.8 hours as opposed to 63 ± 23.5 hours), cleared fever earlier (87 ± 46.1 hours as opposed to 110 ± 79.0 hours), and cleared parasites significantly faster (55.5 ± 27.4 hours as opposed to 103 ± 39.2 hours) than the patients who had received standard treatment. There were 2 deaths out of the 15 patients in the group with the loading dose, and 3 deaths out of the 12 patients in the other group. The plasma quinine concentrations were measured in the patients receiving the loading dose. These findings were compared with those obtained under standard treatment (White et al 1982). In the patients receiving the loading dose, the quinine mean concentration at the end of the first i.v. drip was 40×10^{-6} M (against 21×10^{-6} M with standard treatment); the trough level before the second infusion was 28×10^{-6} M (as opposed to the 14×10^{-6} M expected). Peak concentrations over the first 52 hours rose only slightly until they reached a maximum of 46×10^{-6} M. During this period the troughs became shallower. Until day 3 the quinine levels in the group receiving the loading dose were consistently higher than those estimated from standard treatment. The loading dose permits a significantly more rapid establishment of quinine concentrations above the MIC level. This is of particular importance in patients with cerebral malaria where case fatality occurs mainly within the first 24 or 48 hours, i.e. during a time when 'standard' treatment will often fail in producing drug concentrations above the MIC level.

treatment with a concentration ratio of about 0.5 (erythrocytes/plasma). This began to decrease from the second day after the start of treatment and a ratio of approximately 0.3 was reached by day 6. The elimination half-life of quinine in red cells was consistently shorter than the plasma elimination half-life in patients with cerebral malaria or uncomplicated falciparum malaria as well as in convalescent patients one month after treatment. This study brought further evidence of a prolonged plasma half-life of quinine during acute malaria. The specific red cell kinetics of quinine during acute malaria, pertaining to infected as well as non-infected erythrocytes, may be ascribed to membrane changes. From the data of one particular case treated by White et al (1983c), it appears that the quinine concentration in infected red cells was at least six times greater than that in non-infected erythrocytes.

Recently attention has focused on quinidine, a stereoisomer of quinine, as an alternative to quinine. Although the antimalarial activity of quinidine had been known for a long time (see Phillips et al 1985), its use in malaria treatment did not become popular on account of potential cardiotoxic manifestations. Two reasons are responsible for the renewed interest, namely the wide availability of quinidine in areas where quinine may not be on hand and malaria cases may require treatment (Ris et al 1983; WHO 1984b), and the demonstration that quinidine is more effective against *P. falciparum* both in vitro (Oduala et al 1982) and in vivo (Suntharasamai et al 1984). This may be advantageous in areas where the MIC of quinine is close to or higher than the attainable concentrations in vivo. Phillips et al (1985) treated 14 cases of severe falciparum malaria with quinidine by intravenous infusion, 15 mg (base)/kg body weight as a loading dose and subsequently for a total of 7 days 7.5 mg/kg every 8 hours (replaced by the oral administration of the same dose once the patients were able to swallow the capsules). Two patients died from malaria, the other 12 recovered, but recrudescences were observed in two. Parasite clearance time (49.4 ± 17.8 hours) and fever clearance time (69.5 ± 18.7 hours) were comparable to earlier observations with quinine when a loading dose was employed. Quinidine showed a rather small volume of distribution (1.68 l/kg) and a total clearance of 1.75 ml/min. The mean value for urinary clearance was 0.62 ml/min. The elimination half-life, 12.8 hours (mean), appears to be shorter than that of quinine in acutely ill patients. Electrocardiogram (ECG) changes were common. White et al (1983d) compared ECG effects of quinine and quinidine and found significant differences between these drugs with regard to a prolongation of the QT interval. It will be wise, therefore, to monitor ECG and blood pressure during the treatment with quinidine. At this stage it is not known whether long-acting oral formulations of quinidine (Leizorovicz et al 1984) will be suitable for the treatment of malaria. Such formulations produce relatively stable drug profiles and may be less cardiotoxic than the ordinary ones which yield higher C_p levels (Bunnag et al 1987).

PROGUANIL

Interest in proguanil has been revived but information on the pharmacokinetics of this prodrug and its active metabolite, cycloguanil, is scanty.

Proguanil is rapidly absorbed from the intestine, with a time to peak concentration (tp) of approximately 4 hours; the elimination half-life is approximately 24 hours (WHO 1984b). Moody et al (1980) found a peak serum concentration of 1.10×10^{-6} M at 2.5 hours after the oral administration of 200 mg proguanil to a healthy adult volunteer. The whole blood concentration in the 1.5 hour sample was 1.34×10^{-6} M as compared to 0.87×10^{-6} M in serum. This indicates, roughly, a 2½-fold concentration in erythrocytes as compared to serum. WHO (1984b) quoted a 4–8 times greater proguanil concentration in red blood cells as compared to plasma. However, in the interpretation of the data of Moody et al (1980) it should be considered that the distribution of proguanil to the erythrocytes may not have been complete 1½ hours after dosing, the more so as this was well before tp. Moreover, serum concentrations may be higher than those in plasma due to elution during the clotting process, a phenomenon known to occur with chloroquine.

Little is known about the conversion of proguanil to cycloguanil and the metabolite's kinetics.

prohibits direct bioavailability studies, but the AUC values permit some conclusions to be made. The kinetic parameters obtained by Desjardins et al (1979a) are summarized in Table 51.2. The standard deviations are indicative of a considerable variation within the individual dose groups with regard to AUC and $t_{½}$. Such variations are also notable though less marked with the peak concentrations.

There is no evidence of dose-dependent kinetics. Strong plasma-binding of mefloquine is apparently responsible for the lesser volume of distribution as compared to chloroquine, but the volume of distribution (VD) values of mefloquine are still appreciably high and indicative of marked tissue binding of the drug. Mefloquine is extensively distributed and shows a high affinity for lung, liver and lymphoid tissue (Mu et al 1975; Rozman et al 1978).

Comparison of the absorption lag times following the administration of the tablet formulation and the aqueous solution suggests a marked delay in the absorption of mefloquine from the tablets. Also the absorption rate constant (K_a) indicates significant differences in the rate of absorption from the tablet as compared to the aqueous solution. Desjardins et al (1979a) suspect that probably less than 65% of mefloquine was being absorbed from the tablets used in these studies.

The half-life of mefloquine (mean 13.89 ± 5.31 days) is relatively long, but subject to considerable variation which may be large enough to cause an occasional failure of mefloquine treatment in the absence of true parasite resistance (Desjardins et al 1979a). Elimination of mefloquine follows mainly the biliary route but there is evidence of enterohepatic recirculation.

In a single-dose kinetic study, Schwartz et al (1982) followed the profiles of mefloquine and of one of its metabolites [2,8-bis (trifluoromethyl)-4-quinoline carboxylic acid] in six Caucasian and 13 African male volunteers, using assay methods based on thin layer chromatography and UV densitometry or on gas chromatography/mass spectrography and selected ion monitoring (Schwartz & Ranalder 1981).

After administration of single doses of 250 mg of mefloquine (base) in three Caucasian subjects, the lag time varied between 0.25 and 0.47 hours, the absorption constant K_a was 0.79–1.90, and the elimination half-life $t_{½}$ was 15–24.2 days (mean 19 days). In the three Caucasians who had received 1000 mg mefloquine (expressed as base)

Table 51.2 Single dose kinetics of mefloquine hydrochloride in healthy adult males, according to Desjardins et al (1979a)

Dose p.o. No. of subjects Parameter	250 mg tablet 4 Mean ± SD	500 mg tablet 4 Mean ± SD	1000 mg tablet 4 Mean ± SD	1500 mg tablet 4 Mean ± SD	500 mg solution 4 Mean ± SD
Dose mg/kg	3.45±0.46	6.84±0.66	14.01±2.08	19.61±1.95	6.71±0.38
Peak time (h)					
Estimated	13.3 ± 1.3	16.5 ± 2.6	27.8 ± 4.7	18.5 ± 4.1	8.0 ± 2.8
Measured	13.5 ± 1.0	12.3 ± 1.9	36.0 ± 8.5	16.8 ± 7.6	10.5 ± 1.7
Peak concentration (10^{-6}M)					
Estimated	0.53	0.97	1.71	2.68	1.30
Measured	0.60	1.04	1.93	2.95	1.40
Peak concentration (μg/ml)					
Estimated	0.22±0.07	0.40±0.10	0.71±0.11	1.11±0.30	0.54±0.12
Measured	0.25±0.08	0.43±0.10	0.80±0.13	1.22±0.36	0.58±0.12
Log time (h)	0.40±0.20	0.56±0.22	0.36±0.18	0.36±0.11	0.02±0.01
$AUC_{0\to\infty}$ (μg. ml/day)	3.50±1.40	6.23±1.43	15.56±7.51	18.79±5.45	9.71±3.19
VD (l/kg)	11.97±3.03	11.84±2.90	17.31±1.91	13.23±3.17	12.17±2.70
K_a/day	6.22±3.17	4.26±2.29	3.31±1.08	3.70±0.88	15.34±6.60
$t_{½}$ (day)	12.15±6.33	11.34±1.68	17.56±6.83	14.33±4.96	14.08±5.76

quinolines which possessed antimalarial activity. One of these, 1,4-bis [2-(7-chloro-4-quinolylamino) propyl]-piperazine or 12 278 RP possessed a residual activity of 28 days against blood infection with *P. berghei*. Subsequent studies in man showed this compound to be less well tolerated and to possess a duration of action no greater than chloroquine (WHO 1973). More recently, workers in the People's Republic of China have synthesized this compound along with several derivatives which have been reported to be more active and long-acting than the parent compound (Qu et al 1981). One of these derivatives, hydroxypiperaquine (see also p. 1632), had a residual activity of 30–45 days against *P. berghei* when it was administered at oral doses of 150 mg/kg. Although it is reported that these compounds are active against chloroquine-resistant parasites in laboratory animals (Qu et al 1981) and in man (Guan et al 1981; Y T Li et al 1981a), the latter without problems of tolerance, there does appear to be some degree of cross-resistance between this series of compounds and chloroquine (Canfield, personal communication).

The 2,4-diamino-6-substituted quinazolines have also been shown to have potential as repository formulations. The most potent of these, 2,4-diamino-6-(2-naphthyl)-sulfonylquinazoline or WR 158 122, protected rhesus monkeys from infection by *P. cynomolgi* for five to seven months following a single intramuscular injection. No local or systemic adverse reactions were observed (Thompson et al 1970). The compound is highly active as a blood schizontocide against both chloroquine-sensitive and -resistant strains of the parasite, but its activity is severely compromised by pre-existing resistance to pyrimethamine. In addition, the rapidity with which resistance to the quinazoline develops when administered alone poses another problem, although resistance to WR 158 122 did not arise when it was administered with an appropriate sulfonamide (Schmidt 1979a, b; Schmidt & Rossan 1979).

CHEMICAL MODIFICATION OF DRUGS TO EXTEND DURATION OF ACTION

Chemical modification of existing antimalarial drugs may also result in the formation of repository formulations, e.g. by converting a bioactive base or acid into an insoluble salt with the appropriate properties. This was the basis of the pioneering studies of Thompson and his co-workers at Parke-Davis which led to the development of the pamoate salt of cycloguanil and demonstrated the potential value of these types of compound (Elslager 1969). Cycloguanil pamoate had an extremely long duration of action, affording protection from chlorguanide-susceptible strains of *P. vivax, P. falciparum* and *P. malariae* for minimum periods of four to six months after a single intramuscular injection in man. Unfortunately, the drug had two major drawbacks. Protection was not obtained from chlorguanide-resistant *P. vivax* and *P. falciparum* and local reactions at the injection site, although rarely severe, were troublesome and caused difficulties in injecting the drug under field conditions (Clyde 1968). This led to the abandonment of these compounds and highlighted the difficulties associated with the use of long-acting 'depot' drugs.

The formulation of pamoate salts to extend the action of antimalarial drugs, however, may still be a valid concept. For example, pyrimethamine also forms an insoluble salt with pamoic acid (Elslager & Worth 1966) and this salt as well as others formed from related bis-naphthoic acids protected mice from challenge with *P. berghei* for more than eight weeks. Equally important was the observation that irritation at the injection site was not observed in mice (Elslager 1969). More recently, single intramuscular injections of 50 and 200 mg/kg of pyrimethamine pamoate in benzyl benzoate/peanut oil have protected rhesus monkeys for two and four months respectively against *P. cynomolgi* infections also without local tissue irritation (Worth & Werbel 1984). These results obtained with pyrimethamine as a model drug—the development of an extended release formulation of this drug would no longer be acceptable operationally—indicate the potential of these compounds. However, greater consideration must also be given to the type of 'carrier' to be used with these formulations. In the early experiments pyrimethamine pamoate was dispersed in benzyl benzoate/peanut oil which, although acceptable for use in man, may show variation from batch to batch. As a result formulations of

hypersensitive to sulfonamides, it is foreseen to use mefloquine in malarious areas in combination with sulfadoxine and pyrimethamine (tablets containing 250 mg mefloquine estimated as base, 500 mg sulfadoxine and 25 mg pyrimethamine) provided that the combination offers a therapeutic advantage over mefloquine alone (see also p. 1585/1586). The rationale for this is based on experimental studies in the *P. berghei*/mouse model which showed that the combination of mefloquine with sulfadoxine and pyrimethamine delays the development of mefloquine resistance (Merkli & Richle 1980; Merkli et al 1980; Peters & Robinson 1984a).

Studies in human subjects are of particular importance since the pharmacokinetic parameters of mefloquine, sulfadoxine and pyrimethamine show a great variation between various animal species, thus precluding reliable conclusions with regard to conditions in man. Schwartz & Weidekamm (1982) have studied the pharmacokinetics of sulfadoxine plus pyrimethamine, of mefloquine alone, and of the combination of the three compounds in two volunteers. The results indicate that the half-life and the other kinetic parameters of the individual compounds are not significantly modified when the drugs were given together. Thus one would not expect problems with the bioavailability of the combination, or metabolic interference which could reduce the efficacy of the individual components. The subsequent, wider pharmacokinetic studies confirmed these preliminary findings (WHO 1984b). Moreover, mefloquine and sulfadoxine/pyrimethamine show in vivo at least an additive effect (preserving the potentiation of pyrimethamine by sulfadoxine), an observation borne out by the results of clinical trials of the combination. The introduction of more sensitive and reliable assays such as an improved HPLC method (Kapetanovic et al 1983) and a GC-EC detection technique (Heizmann & Geschke 1984) will facilitate future pharmacokinetic studies of mefloquine.

REPOSITORY DRUGS

The optimal use of antimalarial drugs requires the constant maintenance of effective concentrations of the drug in the blood of an individual. Although several useful drugs are still available which will interrupt the life-cycle at various stages, they are effective only for brief periods and relatively frequent dosing is required, particularly of drugs which are used prophylactically. This has led to immense problems of logistics, costs and patient compliance for the health workers involved in malaria control and eradication. In addition, a fundamental limitation common to all conventional dosage forms is that they release the drug to the body tissues or fluids at rates which vary with time, i.e. the release rates are highest initially and decline thereafter. This leads to high-low blood level variation between each drug administration. Such fluctuations are particularly deleterious when the toxic level is close to that required for therapy. It has been considered for many years that this may be overcome by the development of drugs with a repository or long duration of action. This approach is not a new one (for reviews see Elslager 1969; Peters 1970a) and, although after many years of research there is no presently employed antimalarial drug or formulation which possesses marked residual tissue or blood schizontocidal activity in man, it is still valid. This is especially true with the new technologies of drug formulation now being developed.

There are two basic approaches to the development of long-acting antimalarial drugs. The one is to search for substances which after oral administration are fixed by host tissues from which they are slowly released; the other involves methods for prolonging the effects of known antimalarial drugs. As the duration of action of a drug is dependent on the rate at which it is released from the formulation, absorbed, metabolized and excreted, the duration of action can be extended by altering the rates of any of these processes.

INHERENTLY LONG-ACTING FORMULATIONS

Representatives of several classes of antimalarial drugs persist in the tissues for relatively long periods following oral administration, although none has yet been demonstrated to have useful repository antimalarial activity in man. For example, Schneider et al (1965) used a novel screening technique to evaluate two bis-4-amino-

pyrimethamine and pyrimethamine pamoate have also been made in pure oils prepared from dimeric ethoxytetrahydropyran ester (Et) and its alkyl analogues (Graham & Howells, personal communication). These oils are novel and supposedly non-toxic solvents which appear ideally suited to the formulation of insoluble compounds with characteristics shared by many antimalarial drugs. Their advantage is that they are reported to be biodegradable and can be obtained in quantity and quality whereas other oils, e.g. peanut oil used in the preparation of pyrimethamine pamoate referred to above, vary from batch to batch. The Et oils may also have advantages when emulsified with aqueous solutions and may guarantee a more constant drug release. Comparisons between pyrimethamine base and pyrimethamine pamoate in Et oils and in peanut oil/benzyl benzoate have been carried out in a rodent model. To date, complete protection against challenge with *P. berghei* has been obtained for up to 56 days in mice given 30% pyrimethamine pamoate in both peanut oil and Et, and all mice given pyrimethamine pamoate in peanut oil and three out of five mice given the drug in Et were protected at 70 days.

It remains to be seen whether the principle of formulation of pamoate salts can be applied to extend the action of candidate antimalarials which do not exhibit resistance or cross-resistance to existing antimalarials. If practical, these salts would require formulation in an acceptable carrier. Biodegradable oils appear to have potential for this role, but a great deal needs to be known on their degradation in man, their toxicity and the drug/carrier interactions before their potential can be assessed.

Acylation of amino and hydroxy groups has also been shown to be a way of extending the duration of drugs. This assumes that the active principle of the drug is regenerated by in vivo deacylation which has proved successful with aspirin, N'-acetylgantrisin and N,N'-diacetamidodiphenyl-sulfone (DADDS). Such compounds have different chemical and physical properties and have to be considered as novel drugs. Several N-acetylated derivatives of pyrimethamine have been synthesized (Brossi, personal communication).

Latentiation, a molecular modification method (Harper 1962), is the means by which a biologically active compound is incorporated into an inactive carrier or prodrug. The therapeutic action develops only after biotransformation through enzymatic or non-enzymatic processes. This method, using polymers as the carrier, can be used to prolong drug activity, but there is only one report of the application of this principle to antimalarial drugs. Korolkovas et al (1978) produced prodrugs of dapsone and sulfadimethoxine through covalent bonding of the parent drug to starch polymeric dialdehyde (Sumstar-190). The results suggested that the antimalarial activity of the compounds was increased due to a more prolonged duration of action and better absorption of the formulation. These results require further evaluation.

DELAYED DEGRADATION AND EXCRETION OF ANTIMALARIAL DRUGS

It has been shown that in principle it is possible to prolong the half-life of certain antimalarial drugs by inhibiting their enzymatic degradation. However, pharmacokinetic and metabolic considerations make the approach impractical. No compounds are known to delay the excretion of antimalarial drugs.

SUSTAINED RELEASE FORMULATIONS

The duration of action of drugs can also be extended by incorporating them into biologically 'inert' matrices and thus delaying their release. Such systems, known as sustained, extended or controlled release formulations, are a relatively new development whose concept encompasses many mechanisms. All systems currently under development, however, have at least two common features. All have a drug reservoir which stores the drug in a stable form and in an amount required for the prescribed treatment. In this way the drug is also protected from degradation by the host. In addition, all formulations contain some system for controlling the release of the drug so that a prescribed level is maintained throughout the operational life of the formulation. Such a rate-controller can be based on physical-mechanical principles as shown with an intra-

venous infusion pump or on physical-chemical principles as observed with diffusion or erosion controlled solid dosage forms. The success of this type of formulation depends primarily on the extent to which the various rate-controlling mechanisms can be defined and optimized. Most of the solid dosage forms currently under development for controlled drug release are based on either the diffusion of drugs through rate-controlling membranes (non-biodegradable formulations) or the release of drugs entrapped in erodible polymers (biodegradable formulations). These diffusion-controlled devices can be divided into two main types: reservoir systems in which the drug is totally encapsulated within a rate-controlling membrane and monolithic systems in which the drug is dispersed or dissolved in the rate-controlling matrix. The diffusion rates from such systems follow simple laws of physical chemistry and, since reservoir systems have no time-dependent variables, they exhibit theoretically constant or zero-order drug release. They may be macrodevices which can be inserted into a body cavity or implanted surgically or they can be microcapsules which can be administered with an ordinary syringe or needle. The first reported attempt to prolong the activity of antimalarial drugs by the use of sustained release systems was by Powers (1965) who, using non-biodegradable silicone rubber implants containing pyrimethamine, showed that mice could be protected against *P. berghei* challenge for five days and chickens against *P. gallinaceum* infections for 12 days. These results were not impressive but Fu et al (1971, 1973a, b) subsequently demonstrated that both chloroquine and pyrimethamine were released from silicone rubber implants over a period of several months.

Howells & Judge (1981) have extended such studies using a variety of antimalarial drugs. These authors prepared implants either by sealing powdered drug into short lengths of silicone rubber tubing or by mixing the drug in the required quantities into prevulcanized silicone rubber prior to vulcanization at room temperature by the addition of stannous actoate. No significant extension of the antimalarial action of primaquine, chloroquine, cycloguanil, sulfadiazine, WR 99 210, WR 99 209, menoctone or mefloquine was obtained by the use of such implants. However, tubular implants filled with powdered pyrimethamine base and implants of silastic matrix containing 0.5% (w/w) of pyrimethamine base protected mice from challenge with *P. berghei* for periods of at least three and five to six months respectively. The reason why only the pyrimethamine formulations showed sustained release characteristics is unknown.

Pharmacokinetic studies in vivo and release studies in vitro were not performed, but the authors do suggest that the failure of most antimalarials to exert a sustained release effect was due to the failure of the implants to release sufficient drug for antimalarial action. It is interesting to note that both menoctone and cycloguanil hydrochloride which have been shown to be skin irritants per se also caused skin irritation and gross local tissue reaction when included in either the silicone rubber or silastic matrix implants. The other preparations failed to show gross host reactions.

It is inconceivable that silicone rubber matrices would be employed operationally for antimalarial therapy since such implants would require both surgical introduction and removal at the end of the release phase. This has led certain workers to develop biodegradable implants for antimalarial therapy. The rate of release from biodegradable devices depends on the surface area of the formulation and the rate of degradation of the polymers. The minimum amount of drug is obtained when the rate of erosion of the matrix is greater than or equal to the rate of drug release by diffusion. At this limit zero-order kinetics are obtained, provided the surface area of the formulation does not change during the release period (Wise et al 1979a). Larger biodegradable devices suffer from the disadvantage that the surface area is relatively small and that it changes as the devices are biodegraded. With such systems the rate of drug release decreases substantially with time. Microcapsule formulations, however, have larger total surface areas that change very little until the microcapsules are completely resorbed. Nearly constant zero-order rates can theoretically be achieved with erodible microcapsule systems which therefore appear to have greater potential for malaria chemotherapy.

There are reports in the literature of two biodegradable polymer systems which have been studied in an attempt to develop sustained release formulations of antimalarial drugs. One is based on the use of polyglycolic acid and polylactic acid polymers. The other uses a polymer based on dihydropyrans (3,4-dihydro-2H-pyran-2-methyl (3,4-dihydro-2H-pyran-2-carboxylate)) and glycerol. The former polymer has been used for almost 20 years as synthetic resorbable sutures and is now proving to be useful as a potential erodible matrix and membrane material for a variety of sustained release formulations.

Wise and his colleagues (Wise et al 1976, 1978, 1979b) have used this copolymer as a matrix for the development of injectable formulations of a quinazoline (WR 158 122) and also of sulfadiazine. Using WR 158 122 (2,4-diamino-6-(2-naphthylsulfonyl)-quinazoline), a copolymer of 75% dl-glycolic and 25% dl-lactic acids containing 16.7% or 33.3% drug was prepared in finely divided particles of less than 125 microns in size by spray-drying. Suspensions of this formulation in carboxymethyl cellulose were injected into the scapular region of mice and the mice challenged with *P. berghei*. Antimalarial activity as measured by repeated challenges and by the Rane test was found to be prolonged over a period of more than 14 weeks compared with 12 days when the drug was administered alone. Recovery of excreted radioactivity from labelled drug indicated that a sustained release of the drug or its metabolite(s) was obtained over the 14-week period and that, at the end of the observation period, 2% and 13% of the drug remained respectively at the injection site of the 16.7% and 33.3% drug/polymer formulations (Wise et al 1976).

The most successful formulation for the sustained release of sulfadiazine was a copolymer of l-lactic acid and dl-lactic acid in the ratio of 90% and 10% by weight respectively containing 33.3% (w/w) of the drug. Studies with this formulation indicated that both the molecular weight of the copolymer and size of the particles influenced the release characteristics. A molecular mass of the polymer of 150 000 and a particle size of 1.5 mm was found to be optimum. The injected formulation gave effective protection against repetitive weekly challenges of *P. berghei* for a period of more than 21 weeks although the drug was only released at a constant rate for just over three months, as measured by the appearance in the urine of mice of ^{35}S from radiolabelled sulfadiazine (Wise et al 1978). These two studies indicate that each drug must have a specifically developed individual polymer matrix to obtain optimum release characteristics. This was confirmed by Wise et al (1979b) who attempted to develop a sustained release combination of WR 158 122 and sulfadiazine. The authors, using a polymer matrix of dl-glycolic acid and l-lactic acid (10% and 90% by weight respectively), injected into both mice and monkeys a blend of two preparations, each containing the respective drugs at concentrations of 50% (w/w) and in a weight ratio of ten parts of sulfadiazine to one part WR 158 122. The results indicated that although the release of WR 158 122 was approximately as desired, that of sulfadiazine was much too rapid to allow the development of a pharmacokinetically matched combination. Thus, although it is theoretically possible to match the pharmacokinetics of two drugs to be used in a combination, the development of such combinations may require a great deal of work.

The release characteristics of a polymer/drug composite will also be affected by the molecular weight of the polymer. This was shown by Wise et al (1978) in their studies on the sustained release system for sulfadiazine. However, these studies involved batches of polymer which had 'average' molecular weights and thus were not completely homogeneous. Further subfractionation may give rise to better release characteristics as has been shown by studies on a glycolic/lactic acid polymer composite with pyrimethamine (Wise, personal communication).

To date, only implants of the dihydropyran/glycerol polymers have been reported as possible sustained release formulations for antimalarial drugs. Judge et al (1981) have shown that 50 mg implants of this polymer containing 20% pyrimethamine protected mice against challenge with *P. berghei* for more than 20 weeks and 46.7 mg implants containing 20% sulfadiazine protected mice against the same parasite for 35 weeks. At present, nothing is known regarding the drug release characteristics of these drug/polymer formulations in vitro or the pharmacokinetics of

drug release in vivo. Preliminary toxicological studies indicate that the implants are well tolerated by the host for up to 200 days but the host reaction after this time is unknown.

Whilst these preliminary reports indicate that the development of biodegradable sustained release formulations of antimalarial drugs may be feasible, the techniques are still in their infancy and many questions require to be answered before these formulations can be judged to have operational usefulness. The matrices themselves have to be fully characterized both chemically and physically and their production by methods acceptable for use in man has to be shown to be under control. Although the matrices used in the development of antimalarial formulations are known to be biodegradable, surprisingly little is known of their different stages of degradation or of the end products. The effects of their long-term use in experimental animals and man must also be determined. The decision as to which drug or drugs are to be selected for use in such systems will have to be made in the light of future operational needs and the current spread of multi-resistant strains of *P. falciparum*.

Current work on sustained release systems has generally been carried out with drugs that are no longer applicable for operational use in such systems. It is difficult, however, to suggest at present a drug which would be suitable. Selection of a suitable drug will depend on various factors such as its cross-resistance pattern to other drugs, its compatibility with the matrix selected and its pharmacokinetics and metabolism in the host. Such a drug should have the following characteristics: high antiparasitic activity, activity against current drug-resistant strains, ideally fast excretion and minimal distribution in the tissues. The formulation of a blood schizontocide which would be protective for a period of at least three months may be operationally useful for certain population groups.

Finally, in spite of the obvious advantages of such systems, sustained release formulations may have a potential limitation in that the sustained low-level release of drug might facilitate the emergence of drug-resistant strains of malaria parasites. This is not known but the systems described above, although not necessarily offering operationally useful formulations, do provide a way of answering this question.

SCREENS FOR REPOSITORY FORMULATIONS

In spite of the relative lack of success in developing long-acting drugs, the search for such compounds still continues. This will in part be assisted by the development of a method for the identification of long-acting blood and tissue schizontocides (Schofield et al 1981). This screening technique has been designed to detect compounds which may possess residual causal prophylactic or residual blood schizontocidal activity or both and which may act following either oral or subcutaneous administration.

TARGETING OF DRUGS

A major obstacle to the successful use of many drugs is their side effects and their inability to reach specific targets in the body. With respect to malaria chemotherapy, this is particularly true of the 8-aminoquinoline, primaquine, which is still today the only operationally useful tissue schizontocide and whose operational usefulness is restricted by such problems. Recent advances in cell biology, immunology and other related areas have suggested that these problems may be overcome by the use of carrier systems which will protect the host tissues from the drug which they carry (and vice versa) and, at the same time, guide them to where they are required or facilitate their release at their site of action. In such a system, the drug-carrier complex should preserve its integrity, avoid association with normal cells, penetrate interposing membranes and selectively recognize, and associate with, the target. This should then facilitate both the release of the drug from its carrier and the disposal of the carrier itself.

Medical research, particularly in the cancer field, has shown that macromolecular, cellular and synthetic carrier systems can be developed

(Gregoriadis 1981). At present, however, despite a large amount of published data, the significance of targeting and its impact on medicine is unknown.

Two methods have so far been applied in experimental chemotherapy of malaria, i.e. the use of liposomes and glycoproteins as carriers for targeting of primaquine. Primaquine incorporated into multi-lamellar liposomes, consisting of phosphatidylcholine, phosphatidylserine and cholesterol in the ratio of 4:1:5, has been shown to be about 3.5 times less toxic to mice than the free primaquine. Although drug activity was not enhanced as compared to the free drug, 100% causal prophylactic doses of primaquine (60 and 70 mg/kg body weight) could be administered in a single intravenous injection to treat sporozoite-induced *P. berghei* infections in mice (Pirson et al 1979, 1980a). The encapsulation of primaquine in cholesterol-poor liposomes did result in an increase in drug efficacy but a substantial enhancement of toxicity as compared with cholesterol-rich liposome preparations and did not permit the administration of 100% curative doses in a single injection. Optimal therapeutic activity was observed three hours after sporozoite inoculation, but partially curative activity was observed three hours before sporozoite inoculation. Primaquine entrapped in liposomes was found to dissociate partially on intravenous injection. The plasma levels of the drug fell more slowly than those after administration of free primaquine. The tissue distribution was also changed; the spleen and liver accumulating respectively three and two times more drug following primaquine-liposome injections than from the free drug. This is in agreement with other studies which show a selective uptake of liposomes by the liver and spleen (Kimelberg 1976; Juliano & Stamp 1975). Other tissues such as lung, kidney and heart accumulated between five and 30 times less drug from primaquine liposomes than from free primaquine, which may explain in part the reduced toxicity of the liposome preparations (Trouet et al 1981). Studies on the kinetics of uptake and distribution of free and liposome entrapped primaquine using isolated perfused rat liver indicate that, whereas free primaquine is predominantly accumulated in the hepatocytes, both hepatocytes and Kupffer's cells take up the liposome-primaquine preparations, with a greater specific uptake by Kupffer's cells (Smith et al 1983). This may account in part for reduced toxicity but similar efficacy of liposome preparations compared to the free drug.

Glycoproteins possessing sugar groups which bind selectively to receptors on various cell types have considerable, but largely unexplored, potential in targeting. For instance, the terminal galactose of asialoglycoproteins interacts with a receptor on hepatic parenchyma cells and, in this way, these proteins are taken up by endocytosis. Asialoglycoproteins are therefore likely to be lysosomotropic as drug carriers in a similar way to that of liposomes. Trouet and his co-workers have also explored this method of targeting primaquine. The synthesis of an adequate link between primaquine and glycoproteins, however, is not straightforward. Primaquine can be linked via its free NH_2 group to a carboxylic side chain in the carrier protein. However, this bond is not an ideal substrate for peptidases and the adjacent carrier may cause steric hindrance. Trouet's group resolved this problem by intercalating amino acid or oligopeptide spacers between the drug and the asialoglycoprotein, asialofetuin. During this study, the amino acid and peptide derivatives of primaquine were shown to have very interesting chemotherapeutic properties themselves.

The amino acids leucine (leu) and alanine (ala) were used as the spacers, and leu-primaquine, ala-leu-primaquine and ala-leu-ala-leu-primaquine were shown to be suitable intermediate products for linking primaquine to asialofetuin since they were hydrolysed rapidly by lysosomal enzymes to release free primaquine. However, leu-primaquine was not hydrolysed in the presence of serum while ala-leu-primaquine and ala-leu-ala-leu-primaquine were slowly hydrolysed to leu-primaquine. The toxic and chemotherapeutic activities of leu-primaquine were similar to those of primaquine and so it is likely that leu-primaquine is either active by itself or is hydrolysed to primaquine within the cell. Surprisingly, ala-leu-primaquine and ala-leu-ala-leu-primaquine were reported to be less toxic and more active than leu-primaquine or primaquine itself. Both derivatives were fully curative in mice inoculated with sporo-

zoites of *P. berghei* after a single intravenous injection of 35 mg/kg body weight. It appeared therefore that ala-leu-primaquine and ala-leu-ala-leu-primaquine may act as prodrugs (Pirson et al 1980b; Trouet et al 1981). However, the superior efficacy of the tetrapeptide-primaquine formulation was not observed in tests carried out in the *P. cynomolgi* rhesus monkey model (Anand, personal communication). Asialofetuin has been linked to the tetrapeptide-primaquine by two methods which, however, produce different results. In a first attempt, ala-leu-ala-leu-primaquine was linked to succinylated asialofetuin. This formulation exhibited prophylactic activity (ED_{50} of 22.4 mg primaquine base/kg) when given to mice three hours before inoculation with *P. berghei* sporozoites. The causal prophylactic activity of this preparation compared unfavourably, however, with free primaquine or the tetrapeptidic primaquine.

In contrast, if the terminal amino group of the tetrapeptide-primaquine is succinylated prior to its linkage to the desialylated glycoprotein, the product has characteristics of asialofetuin. Although it has similar prophylactic properties to succinylated asialofetuin coupled to the tetrapeptide-primaquine, its causal prophylactic activity is enhanced and it is more than twice less toxic than free primaquine. This increased activity allowed 100% cure rates to be obtained in mice given 25 mg primaquine phosphate equivalent/kg three hours after sporozoite infection. This compares favourably with the tetrapeptide-primaquine preparations which gave 100% cure rates at doses of 35 mg/kg.

Although such formulations may not be acceptable for use in man, these results are encouraging leads in the development of targeted drugs for antimalarial chemotherapy. However, a great deal of further work has to be done before such experimental studies can be assessed fully and may be translated into operational use. In addition, these methods may not be the only ways of targeting antimalarial drugs to the parasites. The recent development of specific monoclonal antibodies to sporozoites, asexual blood stages and gametes of malaria parasites could lead to their assessment as possible carriers for drug targeting.

Other attempts to reduce the toxicity and increase the pharmacological activity of primaquine are being made by the synthesis of primaquine complexes with increased hydrophilic and hydrophobic characteristics. Such novel compounds can be produced in sufficient quantities and of a purity to warrant efficacy and toxicity testing. Their application to malaria chemotherapy remains to be assessed.

CHEMOTHERAPEUTIC APPROACHES BASED ON PARASITE BIOCHEMISTRY

Nearly 70 years ago Paul Ehrlich first advocated the rational development of drugs by a study of the biochemistry of parasites. He concluded that the complete and exhaustive knowledge of all different chemoreceptors was the sine qua non for success in chemotherapy. Although knowledge of the malaria parasite's biochemistry is neither complete nor exhaustive, there are strong indications that unique biochemical determinants do exist, and that these could provide a basis for the successful development of chemotherapeutic agents.

Attempts have been made in the past to rationalize drug design. Folate biosynthesis and metabolism have been a fruitful area for antimalarial drug development and generally this has been backed by biochemical studies on the appropriate enzyme. However, the results in other areas have been poor for a variety of reasons. One major factor has been the use of inappropriate test systems. For example, the occurrence of a ubiquinone (co-enzyme Q_8) in malaria parasites different from that in their mammalian hosts (co-enzyme Q_{10}) is thought to be the reason for the activity of the naphthoquinones. Early assays of inhibition of respiration were carried out using *P. gallinaceum* but most work was carried out on beef heart mitochondria and correlation with final antimalarial activity was poor (Sweeney et al 1983). Similarly, potential inhibitors of hypoxanthine-guanine phosphoribosyltransferase were tested against the enzyme from mammalian tissue culture cells, but no activity against this enzyme of the malaria parasites in vivo was observed (Piper et al 1980a, b).

The techniques developed for the cultivation of the erythrocytic stages of *P. falciparum* and for the fractionation and isolation of parasite material have removed in part this constraint. Thus, unless large amounts of parasites are required, biochemical and drug inhibition studies for blood schizontocides can now be carried out on the target parasite. The in vitro culture system has also provided a simple and cheap screening system which has led to the identification of many compounds with antimalarial activity. The more recent development for the in vitro cultivation of the exoerythrocytic stages of malaria parasites first for *P. berghei* (Hollingdale et al 1981) and later for *P. vivax* (Mazier et al 1984; Hollingdale et al 1985) may prove to be equally of use in chemotherapeutic research. Monoclonal antibodies and DNA hybridization techniques will also have an increasingly important role to play in the identification of potential chemotherapeutic targets. The difficulties of the production of sufficient quantities of the enzymes from cultured parasites could be overcome by cloning in a suitable vector, the plasmodial genes which code for the particular enzyme. The feasibility of this approach has been shown by Simmons et al (1985) who identified a monoclonal antibody to L(+)-lactate dehydrogenase (EC 1.1.1.27) of *P. falciparum* and subsequently cloned the gene for this enzyme in *Escherichia coli*. These studies indicated that the parasite-specific lactic dehydrogenase was structurally distinct from the host cell enzymes.

BIOCHEMICAL TARGETS

Energy metabolism

Glucose metabolism might seem an unlikely target for malaria chemotherapy since the metabolic route is fundamentally similar in all living cells. However, the fact remains that plasmodial enzymes involved in these pathways can be quite distinct from those of the host. Some 20 years ago Sherman (1966) showed that there was heterogeneity of lactate dehydrogenase in *P. lophurae* and that the active site of the parasite enzyme was quite distinct from that of the host enzyme. This has now been observed in *P. falciparum* (Vander Jagt et al 1981; Simmons et al 1985). To date, there have been no reports on the kinetic properties of any other purified glycolytic enzymes, simply because it has been difficult to grow parasites in large enough quantities for conventional biochemical analyses, and for some unexplained reason the plasmodial enzymes are exceedingly labile. However, detailed characterization should provide evidence for parasite-specific receptors. This may now be possible with the development of appropriate monoclonal antibodies and DNA hybridization technology.

Protein synthesis

It is generally agreed that there are three potential sources of amino acids for the erythrocytic stages of plasmodia: (1) CO_2 fixation; (2) the free amino acid pools of the plasma and red cell; and (3) red cell haemoglobin (Sherman 1979).

CO_2 fixation can only supply a limited amount of amino acids for parasite protein synthesis but, despite this, one of the CO_2 fixing enzymes, i.e. phosphoenolpyruvate carboxylase, identified in *P. berghei*, has never been identified in other eukaryotes (Siu 1967). Despite the abundance of studies which document the increased uptake of amino acids by malaria infected cells, there has been no detailed description of the mechanisms by which it is accomplished. If it is due to parasite-specific carriers, it may be possible to produce protein imbalance by specifically blocking such transport proteins. In this context, it is interesting to note that Kutner et al (1983) have described and partially characterized a new permeability pathway for anion transport in human red cells infected with *P. falciparum*.

There is no estimation of the relative importance of free amino acids and haemoglobin as a source of amino acids for the parasite although it is presumed that the haemoglobin of the red cell is the major source. Parasite-specific proteases have been identified in various malaria parasites. If it is true that malaria parasites obtain the majority of their amino acids by proteolysis of haemoglobin, then it is possible that the suspected parasite-specific cathepsin D and aminopeptidases which have been identified (Charet et al 1980; Gyang et al 1982; Sherman & Tanigoshi 1983) could be specific targets for chemotherapy.

The free amino acids derived from blood plasma or haemoglobin enter the parasite by ill-defined processes. Up to now, it has not been possible to fill this major gap in our knowledge, mainly because of the highly unstable nature of the parasite when it is removed from the host cell. The mechanism of protein synthesis in malaria parasites appears to be typically eukaryotic but this is a poorly studied area in which nothing is known of the specific requirements for synthesis.

Studies on the biology of the parasite and on the antigens responsible for stimulating the host's immune response have led to the identification of several parasite-specific functional proteins in addition to parasite enzymes which are potential targets for antimalarial chemotherapy. One of the best studied of these functional proteins is the histidine-rich protein isolated from the membrane-bound cytoplasmic granules of *P. lophurae* and *P. falciparum*. The nucleotide sequence of the histidine-rich protein gene of *P. lophurae* has been determined and the protein successfully translated in a cell-free system (Wallach & Boeke 1983; Wallach et al 1984; Kilejian et al 1985). Histidine-rich protein genes have also been identified in *P. falciparum*. 2- and 4-fluoro-, iodo-, bromo-, chloro- and cyano-histidines have been synthesized and tested for their antimalarial activity in vitro as inhibitors of parasite growth and of the binding of infected cells, a mechanism which involves the histidine-rich protein (see Chapters 4 & 52). Fluoro- and iodo-histidines were found to have antimalarial activity in vitro (Howard, personal communication). However, these compounds are likely to show systemic toxicity to the host similar to that observed with α-fluoromethyl and difluoromethyl analogues.

Nucleic acid synthesis

Although protein synthesis may be typically eukaryotic, RNA from malaria parasites is typically protozoan having a G+C content of 35% in contrast to that of 65% in the host (Sherman 1979). In addition, studies have now shown that the DNA of *P. falciparum*, like that of *P. berghei*, has a unique base composition of approximately 17–19% G+C (Goman et al 1982; Pollack et al 1982). If the ribosomes and the DNA of the parasite are more than inert structures on which parasite molecules are assembled, then their specific base compositions of parasite RNA and DNA could be suitable targets for drug design.

Purines and pyrimidines necessary for the formation of malaria parasite nucleic acids are derived by two distinct routes. Purines cannot be synthesized de novo but are obtained preformed, preferably as hypoxanthine, by purine salvage pathways similar to those of the host cell. *P. falciparum* isolated from the human erythrocyte has been shown to possess the following purine salvage pathway enzymes: adenosine deaminase (EC 3.5.4.4), purine-nucleoside phosphorylase (EC 2.4.2.1), hypoxanthine-guanine-phosphoribosyl transferase (EC 2.4.2.8) and adenosine kinase (EC 2.7.1.20). Both adenosine deaminase and hypoxanthine-guanine-phosphoribosyl transferase have been shown to be structurally distinct from the host cell enzymes based on several kinetic parameters and their reaction to specific inhibitors (Reyes et al 1982). This purine salvage pathway also requires adenylosuccinate as an intermediate between inosine monophosphate (IMP) and adenosine monophosphate (AMP) for the synthesis of adenosine nucleotides. This pathway is not present in normal human erythrocytes (Webster & Whaun 1981). Two known inhibitors of adenylosuccinate synthase (EC 6.3.4.4) have been studied for their effects on adenosine nucleotide synthesis in *P. falciparum*. Hadacidin but not alanosine blocked the synthesis of adenylates from hypoxanthine (Webster et al 1984). Hadacidin acts directly on adenylosuccinate synthase whilst alanosine requires activation by way of an intermediate anabolite, L-alanosyl-5-amino-4-imidazole carboxylic acid ribonucleotide, formed during de novo synthesis of purines.

In contrast to their dependence on preformed purines, malaria parasites synthesize pyrimidines. The enzymes required for the synthesis of thymidylate have been identified in several malaria parasites and the inhibition of one of these, dihydrofolate reductase, has been shown to be the basis of the action of pyrimethamine and other

antifolates. There is reason to believe that dihydrofolate reductase and thymidylate synthase are a bifunctional complex in malaria parasites, whereas the mammalian enzymes exist separately (Ferone & Roland 1980). It is unknown if these are exploitable differences.

All of the enzymes required for the de novo synthesis of deoxyuridylate (dUMP) have been identified in extracts of *P. berghei* (Hill et al 1981). The possible existence of a complex comprising the first three enzymes of this pathway, i.e. carbamoyl-phosphate synthase (EC 6.3.5.5), aspartate transcarbamylase and dihydroorotase suggest characteristics similar to eukaryotes. However, the fourth enzyme, dihydroorotate dehydrogenase is of great interest. It is sensitive to cyanide, antimycin A and menoctone suggesting that, during the oxidation of dihydroorotate, electrons are fed into a cytochrome chain at the ubiquinone level. Cytochrome oxidase, which is also inhibited by cyanide, is the only cytochrome which has been identified in plasmodia but there is no evidence for a tricarboxylic acid cycle in mammalian malarias. It could be that the prime purpose for an electron transport chain in the blood stages of malaria parasites is its involvement in orotate biosynthesis (see p. 1580). If this is the case, the microaerophilic requirements and the presence of cytochrome oxidase make sense, i.e. oxygen is involved in the synthesis of pyrimidines (Gutteridge et al 1979). Some of the enzymes of pyrimidine biosynthesis have been identified in *P. falciparum*, and shown to differ from those in the host (Gero et al 1981; Rathod & Reyes 1983). The latter study suggests that, in contrast to the mammalian system, *P. falciparum* metabolizes orotate first to the free intermediate orotidine-5'-monophosphate and then to uridylate monophosphate. This area of parasite metabolism obviously is promising for drug development.

Folate metabolism

It is well known that sulfonamides and sulfones interfere with the biosynthesis of dihydrofolate and that inhibitors of dihydrofolate reductase, e.g. pyrimethamine, block the formation of tetrahydrofolate. These are the only drugs for which the mechanism of antimalarial action is known (see p. 1573). It has been generally accepted that the malaria parasite, like bacteria and in contrast to the host, uses para-aminobenzoic acid (PABA) and not folic acid for synthesis of folates. Recently, studies with *P. falciparum* in vitro indicate that both folic acid and PABA interfere with the activity of sulfadoxine.

These results suggest that another metabolic pathway may exist in the parasite by which, in the absence of plasma folates and PABA, the parasite is capable of utilizing red blood cell folate present in the form of polyglutamated 5-methyl tetrahydrofolate (see p. 1572). The origin of the pteridine for folate biosynthesis in the malaria parasite is unknown.

Lipid biosynthesis

Malaria parasites obtain a major portion of their requirements for phospholipids, lysophospholipids, cholesterol, fatty acids and phospholipid precursors by participating in dynamic exchanges with components of the plasma during the turnover of erythrocyte lipids (Sherman 1979). Additionally, both membrane lipids of the host cell as well as the parasite become available for plasmodial lipid biosynthesis by endocytotic feeding of the parasite. Lipid biosynthetic pathways may appear, therefore, unlikely areas for chemotherapeutic action of novel antimalarial compounds. However, parasite specific enzymes which are membrane-bound have been identified. These include phosphatidylserine decarboxylase (EC 4.1.1.65) and phosphatidylethanolamine methyltransferase (EC 2.1.1.17) (Vial et al 1982, 1985).

MICROTUBULES

Several electron-microscopical studies have indicated the presence of microtubules in malaria parasites (Aikawa 1977) but no corresponding biochemical investigations on the nature of these structures have been reported. The antimitotic drug, colchicine, which is known to interfere with

microtubule synthesis and function has been shown to have antimalarial activity in vitro (McColm & Trigg 1980) but not in vivo (Coatney et al 1949a). This is an area which might merit further investigation in the search for novel antimalarial compounds.

PARASITE INVASION OF RED CELLS

Major advances made in understanding the mechanism of invasion of the red cell by the parasite may provide leads for potential chemotherapeutic action. Several studies have produced evidence which strongly suggests that the red cell sialoglycoproteins, glycophorin A, B, and possibly C, are the host cell receptors for merozoites (Howard et al 1982; Pasvol et al 1982a, b; Perkins 1981, 1984a).

In addition, potential receptors for attachment to and invasion of red cells have also been identified on *P. falciparum* merozoites. A doublet of *P. falciparum* proteins with a relative molecular mass (Mr) of 155 000 and 130 000 respectively, localized on the surface of merozoites, has been implicated as a component of a functional receptor (Perkins 1984b). These proteins bind to glycophorin A and B and monoclonal antibodies to them block invasion of red cells in vitro.

Other merozoite-specific proteins have been identified by monoclonal antibodies. One, a glycoprotein of Mr 190 000–230 000 has been identified on the surface of merozoites and schizonts of a variety of malaria parasites (WHO 1984a). This protein which, in *P. falciparum*, has an Mr 195 000 appears to vary antigenically within species and contains both species- and strain-specific epitopes. Its role in invasion is unknown but it appears to be involved in the host's immune response since monoclonal antibodies to it inhibit invasion of red cells. Monoclonal antibodies to proteins of Mr 40 000–90 000 and Mr 100 000–150 000, associated with the paired rhoptry organelles of the merozoite, also block invasion of red cells. These proteins may be involved in the process of invasion leading to vacuole formation (Campbell et al 1984; WHO 1984a). All of these proteins have potential for inclusion in asexual blood stage vaccines and for the rational design of antimalarial drugs.

Until recently, nothing was known of the mechanism of entry of malaria sporozoites into the host cell. This was mainly due to the lack of an appropriate test system. However, this constraint has been overcome by the development of the techniques for in vitro cultivation of the exo-erythrocytic stages referred to above. By using these techniques, it has been shown that sporozoite-specific surface antigens are intimately involved in the invasion process (Hollingdale et al 1983, 1984). Immunity to sporozoites appears to be mediated by the binding of antibody to these sporozoite surface antigens thus inhibiting the parasite interaction with the target cell.

OXIDANT KILLING OF MALARIA PARASITES

The involvement of non-specific factors in the killing of *P. vinckei* and *P. chabaudi* and the possibility of these factors being macrophage products has been suggested by Clark and his co-workers for many years. These studies have now produced leads which may have application to malaria chemotherapy. Recently, this group (Clark & Hunt 1983; Clark et al 1983) has produced new data, supported by parallel studies in other laboratories (Allison & Eugui 1982; Dockrell & Playfair 1983), implicating toxic oxygen radicals in the killing of malaria parasites. Their experiments show that both *P. vinckei* in vivo and *P. falciparum* in vitro are killed when their host red cells are exposed to generators of free oxygen radicals such as alloxan and t-butyl hydroperoxide. Although this was not accomplished without side effects, notably a limited haemolysis, parasite death was not secondary to this phenomenon. These results suggest that infected cells are under oxygen stress and, therefore, are more susceptible to the effects of oxygen radicals. They also suggest that compounds which generate free oxygen radicals may be shown to be suitable antimalarials. This may be of interest in connection with several, mainly plant-derived, candidate compounds which may exert oxidative activity.

Whilst this approach warrants further study, an element of caution should be considered since the possibility exists that there may be narrow margins between efficacy and toxicity in such compounds.

EXPLOITATION OF POTENTIAL BIOCHEMICAL TARGETS FOR DRUG ACTION

The preceding section has outlined some areas of parasite biochemistry and biology which may be potential targets for chemotherapeutic action. This information, provided it is reliable and well documented, can be exploited in several ways for the design of new antimalarial drugs. Traditional approaches should continue to be used. However, since sufficient biochemical information on the potential target molecules of *P. falciparum* could become available, there is hope for a more efficient design of inhibitors or antagonists by new methods which are coming into use. For example, computer-assisted molecular modelling can give valuable information for the design of new drugs (Gund et al 1980). Whilst these systems have not been widely applied to antimalarial drug design, they have proved useful for other projects. Computer programmes are available for generating three-dimensional chemical structures, determining preferred conformations, comparing such structures and, where receptor models are available, fitting them to a receptor site. These capabilities greatly extend the chemist's ability to perceive the relationship between structure and activity of current antimalarial compounds and to calculate the properties of hypothetical drugs before they are synthesized. However, basic structural information must be available before such techniques can be used. This can be obtained from physicochemical and X-ray crystallographic studies of the target molecules. Although these latter techniques have not been applied to biochemical targets in the malaria parasite, much structural information can be obtained by the application of X-ray crystallography when it is carried out with sufficient precision and accuracy. The absolute configuration of compounds and the position of carbon, oxygen and hydrogen atoms can be determined with a high degree of accuracy. Although there are major difficulties with compounds of Mr 1000–2000, it is relatively easy to determine the structures of compounds with an Mr of less than 1000 or to deal with high molecular weight molecules such as proteins and nucleic acids by the use of heavy atoms.

If the reaction mechanism of a target enzyme is known, then it becomes possible to design suicide substrates for selective enzyme inactivation. Such substrates must be structural analogues of the normal substrate containing a latent, hidden function group which is unreactive until the target enzyme uncovers it so that it can react rapidly with the active site (Walsh 1983). True suicide substrates show irreversible inhibition of the enzyme but some compounds, such as the anticancer agent methotrexate, show pseudo-irreversible inhibition of enzyme activity and therefore act similarly to suicide substrates.

The feasibility of this approach is illustrated by α, α-difluoromethyl ornithine, a suicide substrate for ornithine decarboxylase which has been shown by Bacchi et al (1980) to have antitrypanosomal activity in vivo. As malaria parasites synthesize pyrimidines de novo, it has been argued that this biosynthetic pathway along with those for folate and thymidylate synthesis, would be areas where the design of suicide substrates would be fruitful. Preliminary results indicate that D-3-fluoroalanine and 5-ethenylorotate may act as suicide substrates for serine transhydromethylase and dihydroorotate dehydrogenase respectively. Detailed studies in this area with *P. falciparum* may, therefore, be profitable.

NEW CANDIDATE ANTIMALARIALS

There are numerous candidate antimalarial compounds in various stages of development; these candidate compounds are therefore presented in three groups, namely those in an advanced stage of investigation including clinical trials, those in advanced preclinical development, and finally other compounds of interest. The borders between these groups are not always well defined,

e.g. in the case of certain naphthoquinones and dihydroacridinediones, but this will be of little importance since the chemical groups and individual compounds are covered in detail.

CANDIDATE ANTIMALARIALS IN AN ADVANCED STATE

Four compounds or chemical groups are covered in this category, the 9-phenanthrenemethanols (halofantrine), the sesquiterpene lactones, pyronaridine and enpiroline. Although the preclinical data on sesquiterpene lactones and pyronaridine are still inadequate, the compounds have been included in this section since they have already undergone extensive clinical studies.

9-phenanthrenemethanols

During the Second World War several 9-phenanthrenemethanols were found to possess blood schizontocidal activity and compounds SN 8867 and SN 9160 were successfully tested in man (Wiselogle 1946; Coatney et al 1947; Arnold et al 1973). However, at the time, these candidate drugs were not explored further since the 4-aminoquinolines were more active and easier to use. Only some two decades later, after the advent of chloroquine resistance in *P. falciparum*, was the exploration of these compounds resumed in the course of the antimalarial drug research programme of the Walter Reed Army Institute of Research.

The first compound to be investigated was 6-bromo - α - (diheptylaminomethyl) - phenanthrenemethanol, WR 33 063 (Fig. 51.2), which had been synthesized by May & Mosettig (1946). After satisfactory preclinical observations, WR 33 063 was used in clinical studies (Arnold et al 1973; Canfield et al 1973; Segal et al 1974; Hall et al 1975a) which demonstrated that the drug was well tolerated in therapeutic doses and rapidly effective in falciparum and vivax malaria, including infections with chloroquine-resistant *P. falciparum*. It was, however, a poor suppressant (Clyde et al 1973), probably due to its rather short half-life. Since reliable clinical treatment with WR 33 063 required the administration of 1.6 g (in four divided doses) daily for six days, the search for other 9-phenanthrenemethanols was extended (Chien et al 1972; Washburn & Pearson 1974; Markovac & LaMontagne 1976) and two compounds, WR 122 455 and WR 171 669, were found to be more effective than WR 33 063 in experimental malaria. WR 122 455 is the hydrochloride of α-(2-piperidyl)-3,6 bis (trifluoromethyl)-9-phenanthrenemethanol (Fig. 51.3); and WR 171 669 is the hydrochloride of 1-(1,3-dichloro-6-trifluoromethyl-9-phenanthryl)-3 di(n-butyl)aminopropanol (Fig. 51.4). Although WR 122 455 is more active than WR 171 669 in experimental malaria, including induced *P. falciparum* infections in *Aotus trivirgatus* (Schmidt et al 1978a), tolerance of WR 122 455 in man is less than that of WR 171 669. A reliable treatment of both chloroquine-sensitive and chloroquine-resistant falciparum malaria requires the administration of WR 122 455 over several days (Rinehart

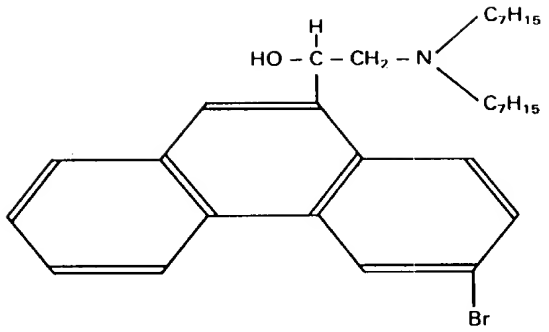


Fig. 51.2 WR 33 063

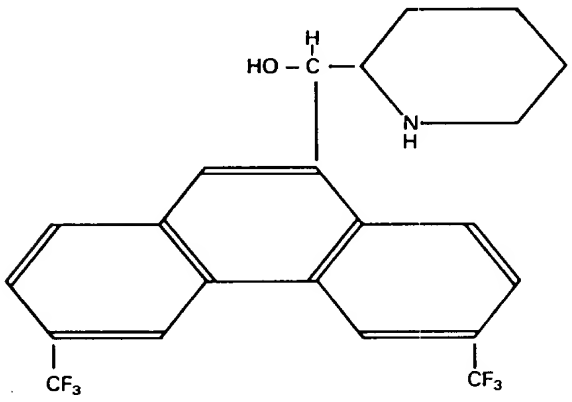


Fig. 51.3 WR 122 455

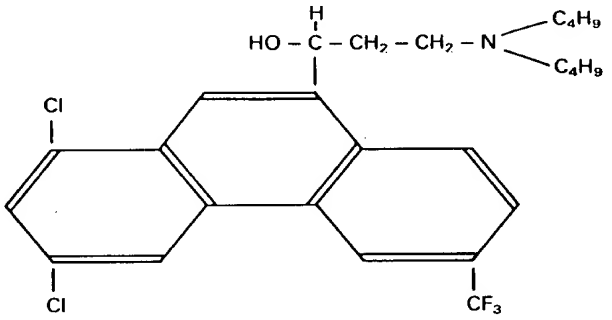


Fig. 51.4 WR 171 669

et al 1976), whereas single-day treatment regimens may suffice with WR 171 669 (Cosgriff et al 1982). In consequence, WR 171 669 was selected for further preclinical and clinical development, and has been given the generic name of 'halofantrine'.

Animal and in vitro studies with 9-phenanthrenemethanols

In the *P. berghei*/mouse model the median curative dose, CD_{50}, of halofantrine was 15 mg/kg, as compared to 54 mg/kg with mefloquine, both drugs being given by the i.m. route (Canfield 1980). The CD_{50} values were quite different in the *P. falciparum*/*Aotus trivirgatus* (owl monkey) model, with 58.2 mg/kg for halofantrine and 7.3 mg/kg for mefloquine. The CD_{90} for halofantrine in the *P. berghei*/mouse system was 30 mg/kg; it was 90 mg/kg in owl monkeys infected with the pyrimethamine-resistant Malayan Camp (MC-CH/Q) isolate of *P. falciparum*; and it was 105 mg/kg in the same model using the chloroquine-resistant Vietnam Oak Knoll (VnOK) isolate of *P. falciparum* (Schmidt et al 1978a). In vitro, using a multidrug-resistant isolate of *P. falciparum*, the median inhibitory dose, ID_{50}, of halofantrine was 7.27×10^{-9} mol/l and that of mefloquine was 18.82×10^{-9} mol/l (Desjardins et al 1979b). At dose levels used in human studies, halofantrine showed no significant toxicity in animals (Cosgriff et al 1982). There was no indication of phototoxicity, a phenomenon which has disqualified quite a number of 9-phenanthrenemethanols with antimalarial activity from further preclinical and clinical development. The administration of 60 mg/kg halofantrine daily to dogs over a period of 28 days was lethal. At these very high dose levels the drug caused weight loss and multiple organ damage, especially to bone marrow and to lymphoid and skeletal muscle tissue in dogs and rats. BUN, SGOT and SGPT were elevated (Cosgriff et al 1982).

Studies of Peters & Porter (1976) on WR 122 455 indicate that this compound is a very active blood schizontocide against *P. berghei* parasite lines resistant to primaquine, sulfonamides, pyrimethamine and cycloguanil. It was also effective against the moderately chloroquine-resistant NS line, but inactive against the highly chloroquine-resistant RC line. Resistance to WR 122 455 was fairly easy to induce in the N and NS lines and was accompanied by cross-resistance to quinine, but chloroquine sensitivity was maintained in these lines. It is possible that halofantrine behaves in a similar way, since its action resembles that of quinine. Canfield (1980) reported cross-resistance between mefloquine and halofantrine in *P. berghei* which had been made mefloquine-resistant through serial passage in mice under drug pressure. However, *P. falciparum* with a reduced sensitivity to mefloquine showed normal in vitro response to halofantrine (Cosgriff et al 1982), confirmed by investigations using clones (Desjardins, personal communication). In an endeavour to define further the structure-activity relationships, 17 selected 9-phenanthrenemethanols were investigated by Childs et al (1984) for their in vitro activity against *P. falciparum*. The findings were compared with those obtained in the *P. berghei*/mouse model after subcutaneous and oral administration and those observed by Schmidt et al (1978a) in the *Aotus*/*P. falciparum* system after oral administration. In 15 out of the 17 compounds the ID_{50} values were lower than those of mefloquine. There was no indication of cross-resistance with chloroquine since the ID_{50} values with the chloroquine-resistant Smith isolate were consistently lower than with the chloroquine-sensitive Camp isolate. Compounds with an alkylaminoalkyl substitution at the 9-methanol were generally more active in vitro than those with a 2-piperidyl or methyl 2-piperidyl substituent. The latter, however, proved to be more active in the *Aotus*/

P. falciparum model after oral administration. The same was found in the *P. berghei*/mouse system after oral administration, whereas the alkylaminoalkyl-substituted compounds were the more active when the candidate drugs were administered subcutaneously. These observations suggest bioavailability problems with orally administered alkylaminoalkyl-substituted 9-phenanthrenemethanols.

The studies of Childs et al (1984) have also demonstrated significant differences in the in vitro activities of two diastereoisomers WR 165 355 and WR 122 455, the most active compounds in the *Aotus*/*P. falciparum* and mouse/*P. berghei* systems after oral administration.

Studies with halofantrine in man

In a Phase I trial in volunteers, Rinehart et al (1976) observed that single oral doses of up to and including 750 mg halofantrine caused no side effects. Similarly, 420 mg three times daily for one day were well tolerated, but mild abdominal cramps or nausea occurred when this dose regimen was extended to two or three days. A regimen with 250 mg every six hours started to produce epigastric pain on the fifth day. There was no gastrointestinal blood loss. In a Phase II study for antimalarial activity, halofantrine was given at a dose of 250 mg every six hours for three days (12 doses) to six subjects infected with the chloroquine-resistant Vietnam Smith isolate, and to three subjects infected with the chloroquine-sensitive African Uganda I isolate of *P. falciparum*. In all nine subjects parasites and fever were rapidly cleared and radical cure was obtained (Rinehart et al 1976). Cosgriff et al (1982) conducted a Phase II study in 27 volunteers who were infected with the Vietnam Smith isolate of *P. falciparum*, using halofantrine in various dose regimens, i.e. 250 mg every six hours for three days (total 12 × 250 mg), 250 mg every six hours for two days (total 8 × 250 mg), 250 mg every six hours for one day (total 4 × 250 mg), 2 × 500 mg in one day with a 12-hour interval, a single dose of 1000 mg or 1500 mg, and a one day treatment with 1000 mg as the first dose, followed six hours later by 500 mg. All regimens with divided doses extending over one, two or three days were radically curative. Recrudescences were seen only in the non-immune groups receiving single-dose treatment (1000 or 1500 mg). Response was relatively quick with mean parasite and fever clearance times of 49 and 73 hours. Three more volunteers, one infected with the chloroquine-resistant Cambodian Buchanan isolate of *P. falciparum* and two with the Chesson isolate of *P. vivax* responded well to halofantrine in doses of 250 mg every six hours for two and three days respectively. Apart from mild gastrointestinal side effects in four persons, the drug was well tolerated and no pathological findings were recorded in the extensive haematological and clinical chemical investigations.

In subsequent field studies in Thailand (Boudreau et al as quoted by WHO 1984b), a loading dose of 1000 mg, followed six hours later by 500 mg, was used and produced cure in only 13 out of 20 volunteers with naturally acquired falciparum malaria. However, with the same total dose given in three doses of 500 mg at 6-hourly intervals, 28 out of 29 infections were cured.

Prospects

Halofantrine is obviously an effective and well tolerated blood schizontocide, permitting the radical cure of falciparum malaria in a one-day, divided-dose regimen. It showed high efficacy in infections with the Vietnam Smith isolate of *P. falciparum* which is relatively resistant to quinine; further development of the compound will depend on the definite determination of the patterns of cross-resistance with mefloquine and quinine since these drugs are, at present, the alternative medicaments of choice for the treatment of chloroquine- and pyrimethamine-resistant falciparum malaria. Current clinical trials in endemic areas should clarify this point.

A problem of bioavailability obviously exists with halofantrine. It is undoubtedly due to poor absorption. The development of a suitable galenic formulation is therefore of high priority.

Sesquiterpene lactones

Chinese scientists in 1971 isolated a fraction with antimalarial activity from the indigenous plant Qinghao, *Artemisia annua* L. (China Cooperative

Research Group on Qinghaosu and its Derivatives as Antimalarials (CCRG) 1982a). The plant, a composite, grows widely throughout China and its value as a herbal medicine has been known for more than 2000 years. The first record of its use dates back to 168 BC and is contained in the 'Recipes for 52 kinds of disease' which were found in the Mawangdui Han Tomb, Changsa, Hunan Province, China. In these recipes Qinghao was mentioned as a remedy against haemorrhoids; its first recorded use as an antimalarial dates from 340 AD during the Eastern Jin Dynasty, when Ge Hong included it in his Handbook of Prescriptions for Emergency Treatments (CCRG 1982a). Qinghao was also included in Li Shizhen's Compendium of Materia Medica which was published during the Ming Dynasty in 1596, indicating that compound Qinghao preparations relieved malarial chill and fever.

Structure and characteristics

From the crude antimalarial fraction of Qinghao, a pure sesquiterpene lactone was obtained and identified as the active principle. The structure and stereochemistry of this compound, named Qinghaosu, also called by the Chinese scientists artemisinin (or arteannuin), were characterized in 1972 (CCRG 1982a). Pending the acceptance of an International Nonproprietory Name, the substance will be referred to in this text as Qinghaosu.

The structure (Fig. 51.5) was established on the basis of infrared spectrum, X-ray diffraction, and ^{1}H and ^{13}C nuclear magnetic resonance. The presence of the peroxide group was confirmed in a quantitative reaction with triphenylphosphine. The lactone structure was ascertained through the colour reaction with hydroxylamine hydrochloride and alcalimetric titration. The structure of Qinghaosu bears no resemblance with any of other known antimalarial drugs or earlier candidate compounds.

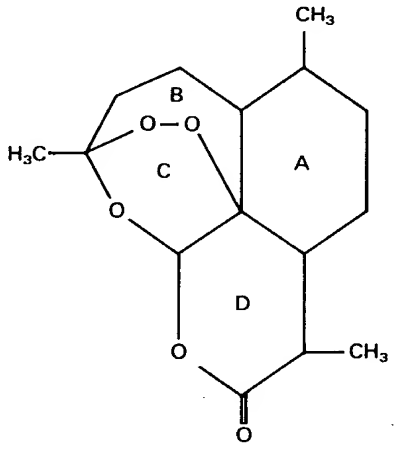


Fig. 51.5 Qinghaosu (Artemisinin)

Qinghaosu crystallizes in colourless, orthorhombic needles with a melting point of 156–157°C. Its molecular formula is $C_{15}H_{22}O_5$ (Mr 282.34). The substance is practically insoluble in water and sparingly soluble in oils, but it dissolves in ethyl alcohol and DMSO. Although the peroxide structure would on first sight suggest that Qinghaosu may be a very unstable molecule, it is actually relatively stable. This stability may be ascribed to its molecular configuration. Both rings B and C, apparently the least stable, are saturated oxyheterocyclic rings. All five oxygen atoms are, sterically, on the same side of the molecule, with alternating short and long C-O bonds. This may account for the unexpected stability (Liu et al 1978; Qinghaosu Research Group 1980; WHO 1981; CCRG 1982a).

Once the antimalarial activity of Qinghaosu had been ascertained and quantitatively assessed, attempts were made to identify the group(s) responsible for the antimalarial activity; it was found that the peroxide structure appeared to be an essential element and that dihydroqinghaosu (Fig. 51.6) was more active, yet less stable than the parent compound. Dihydroqinghaosu was therefore used as the principle for the preparation of more active derivatives with a better solubility. More than 50 esters, ethers and carbonates were obtained in the process (CCRG 1982b) many of which were more active than the parent compound in the *P. berghei*/mouse model.

Among these derivatives, two were chosen by the Chinese scientists for further preclinical and clinical development as they were considerably more active and more soluble. One, artemether, the methyl ether of Qinghaosu (Fig. 51.7), is soluble in lipids such as tea seed oil. The other, the sodium succinyl salt of Qinghaosu, compound 804-Na or sodium artesunate (Fig. 51.8), is readily

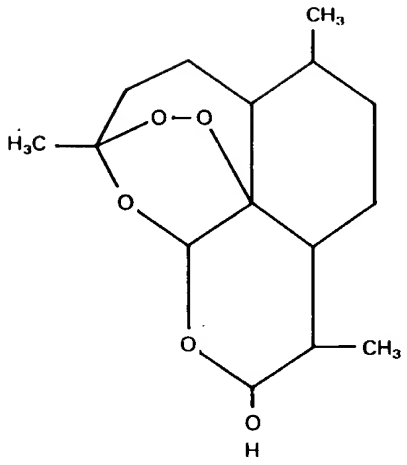


Fig. 51.6 Dihydroqinghaosu

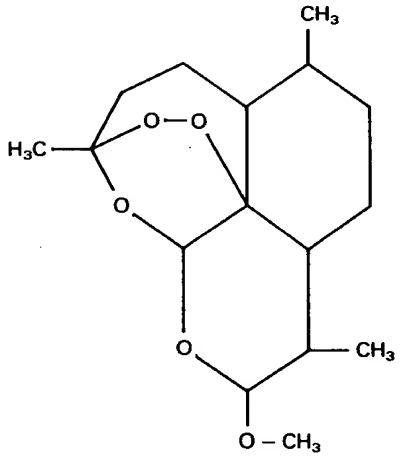


Fig. 51.7 Artemether

soluble in water, but rather unstable in aqueous solution. Sodium artesunate is highly hygroscopic, posing major problems in the formulation of the compound. The free acid (artesunic acid or Qinghaosu succinic acid) is not hygroscopic, and is as effective as the sodium salt.

It is to be expected that Qinghaosu or its derivatives may become drugs for the treatment of malaria, especially of forms requiring rapid medication such as hyperacute or complicated falciparum malaria. However, the final selection of a candidate analogue will probably be made on the basis of the structure/activity relationships

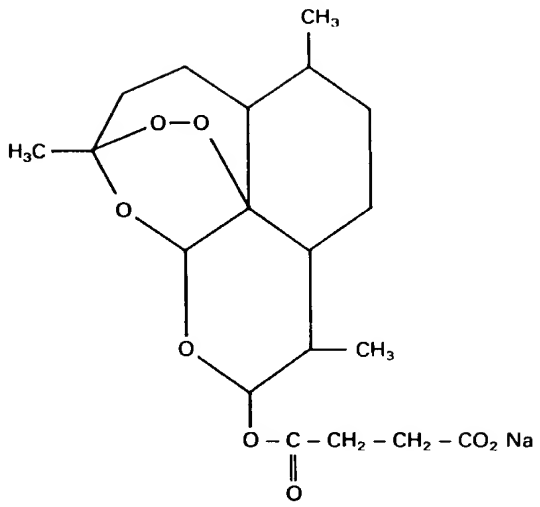


Fig. 51.8 Na-artesunate

already elucidated by Wu & Ji (1982), and of the economics of the synthesis of derivatives using Qinghaosu isolated from the plant. The selection will also be influenced by the envisaged use of the drug. A compound lending itself to the formulation for i.m. injection in relatively small volumes is apt to receive preference, as it would facilitate emergency treatment of malaria at a relatively low level of the primary health care system.

Animal and in vitro studies

The SD_{50} and SD_{90} (SD = suppressive dose) of Qinghaosu, artemether and sodium artesunate were determined by comparison with chloroquine in hybrid Shanghai mice intraperitoneally inoculated with 5×10^6 *P. berghei*-infected erythrocytes (CCRG 1982c), using a chloroquine-sensitive isolate. The drugs were given on days 1, 2 and 3, in one daily dose. The blood was examined on day 4. The results shown in Table 51.3 indicate that artemether in oil solution had the highest dose efficacy, and the least proportional difference between SD_{50} and SD_{90}. The marked difference between the activity of the water suspension and the oil suspension of Qinghaosu, both given by the i.m. route, is apparently due to its solubility and absorption characteristics. The difference

Table 51.3 Antimalarial activity of Qinghaosu and its derivatives in mice infected with chloroquine-sensitive *Plasmodium berghei* (based on data from CCRG 1982c)

	SD_{50} mg/kg	SD_{90} mg/kg	Ratio of SD_{90}:SD_{50}
Qinghaosu, water suspension, i.g.	10.80	28.30	2.62
Qinghaosu, water suspension, i.m.	4.90	8.01	1.63
Qinghaosu, oily suspension, i.m.	0.77	2.15	2.79
Artemether, oily solution, i.m.	0.37	0.53	1.43
Na-artesunate, water solution, i.m.	0.54	1.77	3.28
Na-artesunate, water solution, i.v.	0.94	3.10	3.29
Chloroquine, water solution, i.g.	1.85	2.60	1.41
Chloroquine, water solution, i.m.	0.60	1.12	1.87
Chloroquine, water solution, i.v.	0.67	1.25	1.87

of activity of the water suspension given by the intragastric or the intramuscular route may indicate poor gastrointestinal absorption or degradation before absorption, but it is more likely to be due to a first pass effect. Using the same *P. berghei* isolate and treating the mice once a day for three days as soon as parasitaemia had reached 5 ± 2%, Qinghaosu, artemether, sodium artesunate and chloroquine were given in order to assess equi-effective doses and the speed with which parasitaemia was reduced. Qinghaosu and its derivatives all cleared parasitaemia faster than chloroquine, with sodium artesunate exhibiting the fastest effect but the highest incidence of recrudescences (CCRG 1982c).

Qinghaosu and artemether proved to be highly effective also in mice infected with a chloroquine-resistant isolate of *P. berghei*, but there was a difference in the dose response between the chloroquine-sensitive and the chloroquine-resistant isolate. The resistance index, i.e. the ratio between ED_{50} or SD_{50} levels of resistant and sensitive isolates was 3.6 for Qinghaosu (water suspension, intragastric), 1.7 for artemether (oily solution, i.m.) and sodium artesunate (water solution, i.v.) against an index of 52 for chloroquine.

Qinghaosu has also been tested independently by the Walter Reed Army Institute of Research in a rodent model and against *P. falciparum* in vitro. In the *P. berghei* schizontocidal test it was inactive after oral as well as subcutaneous administration of up to 80 mg/kg, the highest tested dose. However, it was effective subcutaneously in suppressive testing against both the chloroquine-sensitive and -resistant lines (SD_{90} 22 mg/kg per day against the sensitive strain and 31 mg/kg per day against the resistant strain). In vitro against *P. falciparum*, it was effective against both the Camp and the Vietnam Smith strains with an EC_{50} of 0.42 and 0.23 ng/ml respectively (Klayman et al 1984a). Thus, no cross-resistance with chloroquine was observed. These results agree with those reported by Chinese scientists and indicate that the drug is poorly active orally.

Macaca mulatta, intravenously infected with blood stages of *P. cynomolgi*, were given Qinghaosu and artemether at various dose levels for three days after parasitaemia had reached full patency. Qinghaosu, administered i.m. as an oily suspension, produced cure at 20 mg/kg body weight once daily for three days; all animals treated with 10 mg/kg or less showed recrudescences. With artemether, administered i.m. as an oily solution, the dose of 8 mg/kg daily for three days proved to be curative; 4 mg/kg was not always curative and recrudescences occurred in all animals having received a lesser dose. Sodium artesunate (water solution i.v.) acted very quickly and was radically curative in *P. knowlesi*-infected *Macaca mulatta* when given at a daily dose of 6 mg/kg or more for three days (CCRG 1982c).

Qinghaosu had no effect against the exoerythrocytic stages in sporozoite-infected chickens (*P. gallinaceum*), mice (*P. yoelii yoelii*) and rhesus monkeys (*P. cynomolgi*).

Qinghaosu proved to be parasitocidal at concentrations $\geq 10^{-7}$ mol/l when tested in vitro according to the technique of Richards & Maples (1979), using the FCC1 and FCC2 isolates of *P. falciparum* from Hainan (CCRG 1982c). In

vitro, sodium artesunate proved to be the most active compound of the Qinghaosu group. Similar results were obtained by Guan et al (1982) who tested Qinghaosu, artemether and sodium artesunate against the FCC1 isolate of *P. falciparum* from Hainan using a 48-hour in vitro microtechnique and observed EC_{50} values of 7.05×10^{-9}, 7.36×10^{-9} and 0.35×10^{-9} mol/l respectively.

The acute toxicity of Qinghaosu has been assessed in mice, rats and dogs (CCRG 1982d). The LD_{50} of Qinghaosu administered orally in mice was 4228 mg/kg while that of oral chloroquine was 400 mg/kg. The chemotherapeutic indices (LD_{50}/SD_{50}) of both drugs given by the oral route were 384 for Qinghaosu and 216 for chloroquine. The difference was more marked when the drugs were given by the i.m. route, showing an LD_{50} of 3840 mg/kg and a chemotherapeutic index of 4987 for Qinghaosu. Chloroquine given i.m. yielded an LD_{50} of 63 mg/kg and a chemotherapeutic index of 95. The LD_{50} for Qinghaosu in the rat was 5576 mg/kg if given orally, and 2571 mg/kg if given i.m. Dogs having received single i.m. doses of 800 and 400 mg/kg Qinghaosu showed some transient toxic effects which were more marked with the higher dose. While these signs subsided quite rapidly, there was a significant drop in the reticulocyte count some 48 hours after treatment. Acute toxic symptoms induced in several animal species through the i.m. injection of high doses of micronized Qinghaosu consist of restlessness, tremor, slow respiration, motor incoordination, delayed sensation and later on inhibited activity. Larger laboratory animals developed clonic and tonic convulsions, and episthotonus. Normally, these symptoms resolved completely within 10–24 hours. In the animals that died from the intoxication, respiratory arrest always preceded cardiac arrest (CCRG 1982d).

The acute toxicity of repeated administration of Qinghaosu has been assessed in rats receiving 600, 400 and 200 mg/kg daily for seven days (i.m., oil suspension). All animals survived. Apart from some degenerative signs in heart, liver, spleen, lung and kidney tissue in the groups receiving 600 and 400 mg/kg, there were no major symptoms or lesions. Dogs having received 100 mg/kg Qinghaosu for five consecutive days showed no pathological changes at all (CCRG 1982d). Subacute toxicity tests with Qinghaosu administered in daily intragastric doses of 250, 500 and 1000 mg/kg for 14 days failed to cause any pathological changes. In *Macaca mulatta* Qinghaosu was given i.m. in peanut oil for 14 days at daily doses of 24, 48, 96 and 192 mg/kg (CCRG 1982d). The highest dose caused the death of three out of four animals. Toxic symptoms consisted of reduced appetite, apathy, bradycardia and decreased activity. Haematocrit, red blood cell count, and haemoglobin decreased sharply and reticulocytes disappeared. The white blood cell count and the proportion of neutrophils also dropped and the bone marrow showed a marked depression, especially of erythropoiesis. Histologically the cardiac muscle showed cytoplasmic coagulation and mitochondrial swelling, the epithelial cells of renal tubules showed slight cloudy swelling; vacuolar degeneration and accumulation of glycogen was seen in hepatocytes. The next to highest dose, 96 mg/kg for 14 days, also provoked severe toxic effects similar to those of the higher dose schedule, and one out of six monkeys died. In the group receiving 48 mg/kg during 14 days no pathological changes were seen except for a decrease of red blood cell count, haemoglobin and haematocrit as well as a disappearance of reticulocytes and an increased erythrocyte sedimentation rate. The bone marrow appeared to be normal. The 24 mg/kg group showed only a decrease of the reticulocyte count. In spite of their severity the myocardial lesions observed under the high dosages were apparently reversible, as shown on post-mortem material obtained five weeks after the last dose (Wang & Liu 1983).

Tests for muscle irritation indicated no noticeable local injury following the i.m. injection of Qinghaosu in oil or water suspension (CCRG 1982d). Mutagenicity studies using the murine bone marrow polychromatic erythrocyte micronucleus test and the Ames test produced no evidence of a mutagenic effect of Qinghaosu (G Y Li 1981; CCRG 1982d).

Teratogenicity studies were conducted with Qinghaosu administered orally to pregnant Wistar rats at a daily dose of 1/400, 1/200 or 1/25 of the LD_{50} for six days. At 1/25 LD_{50} all living foetuses developed normally and without any deformation except for umbilical hernia in 6.1% of

the rat foetuses when the drug was given during the first six days of gestation. No foetus survived at this dose level when Qinghaosu was given during mid or late gestation (CCRG 1982d), nor did any foetus survive at the dose level of 1/200 LD_{50}, while about half the foetuses were absorbed when 1/400 LD_{50} was given on days 6–15 of pregnancy. Similar results were obtained in pregnant mice.

Toxicity studies on artemether are still quite scanty. The LD_{50} of artemether in mice, treated i.m., is 263 mg/kg, the chemotherapeutic index is 447 (CCRG 1982d). Rabbits tolerated single doses of 160 mg/kg well. Subacute toxicity studies in rats receiving i.m. doses of 40–360 mg/kg artemether for 9–14 days showed no pathological changes except for a slight fatty degeneration of hepatocytes. Similar results were obtained in dogs. Artemether causes no local irritation at the injection site. Teratogenicity studies with artemether (Chen et al 1984) showed no adverse effects on the foetus in mice receiving up to 5.4 mg/kg body weight i.m. daily on days 6–15 of gestation. At a daily dose of 10.7 mg/kg 30% of the embryos were resorbed. All surviving foetuses were normal. In rats receiving 21.4 mg/kg artemether daily on days 9–11 of gestation all embryos were resorbed. Similar results were obtained in rabbits which, like mice and rats, showed no teratogenic effects.

Data on the experimental toxicity of sodium artesunate (CCRG 1982d; Yang et al 1982) indicate an LD_{50} in mice of 520 mg/kg for i.v. injection and of 475 mg/kg for i.m. injection as well as a chemotherapeutic index of 1733 with chloroquine-sensitive *P. berghei* and 1040 with the chloroquine-resistant isolate. The critical level for toxic manifestations in dogs seems to be approximately 75 mg/kg. Studies of the cardiovascular effects in guinea pigs and rabbits showed a relatively low toxicity of sodium artesunate as compared to chloroquine. Subacute toxicity tests with 10 and 40 mg/kg daily for 14 days in dogs produced no pathological changes in the physiological and biochemical parameters. Similarly there were no pathological changes in *Macaca mulatta* treated with 10 and 32 mg/kg sodium artesunate for 14 days. Gross and microscopic examination yielded no signs of irritation at the site of injection (i.m. or i.v.) of sodium artesunate in rabbits, dogs and monkeys. High concentrations of sodium artesunate were found to be immunosuppressive using mouse spleen cells and human peripheral lymphocytes in vitro. There was no sign of immunosuppression in C57 black mice injected with 0.25 g/kg sodium artesunate, whereas an i.p. dose of 1.59 g/kg caused a marked suppression of antibody formation and of cell-mediated haemolysis.

Clinical studies

Qinghaosu has been used in clinical studies since 1973; this is comparatively early considering that most of the above-mentioned toxicity studies were not yet undertaken at the time. Between 1973 and 1980 a total of 3368 cases of falciparum and vivax malaria were treated with Qinghaosu, artemether or sodium artesunate under the auspices of the CCRG in Yunnan Province, Hainan Island and Henan Province (CCRG 1982e). Clinical studies on falciparum malaria were mainly undertaken in Yunnan Province and Hainan Island where *P. falciparum* is predominantly chloroquine-resistant, with a substantial proportion of RII and RIII responses. Table 51.4 shows the distribution of the cases according to type of infection and treatment. All patients with falciparum malaria included in the studies were symptomatic and had an asexual parasitaemia of > 500 parasites/μl. The results were classified as S or R type responses.

In the groups receiving Qinghaosu orally in the form of tablets (one to three doses per day over three days), the mean times for fever disappearance and asexual parasite clearance as well as the radical cure rate were dependent on the total dose. There was no radical cure at a total dose of 2.5 g, whereas roughly half the patients showed radical cure after a total dose of 5 g. Qinghaosu in oil solution and oil suspension, injected once daily during three consecutive days, produced nearly 90% radical cures at total doses of 0.9 g. At lower total doses the cure rates were less. However, mean times required for fever subsidence and parasite clearance were similar in all dosage groups. Both fever and parasite clearance times were prolonged when Qinghaosu was given i.m. as a water suspension (total dose 1.2 g given over three days), but the radical cure rate was

Table 51.4 Clinical trials of Qinghaosu and its derivatives in China 1973–80. (CCRG 1982e)

	P. falciparum	*P. vivax*	Total
Qinghaosu preparations	588	1511	2099
Artemether (oil solution)	829	259	1088
Sodium artesunate (water solution)	181	—	181
Total	1598	1770	3368

87%. Artemether oil solution, administered in one i.m. injection per day over three days, produced radical cure in 88–94% of all patients given total doses of 0.32–0.64 g, but in only 71% at a total dose of 0.24 g. Fever and parasite clearance times were not dose-dependent. The sodium artesunate group received 200 mg on the first day and 100 mg each on the second and the third day (i.m. or i.v.). Fever subsided on average within 26.1 hours, and the mean parasite clearance time was 28.5 hours. However, recrudescences were frequent and the radical cure rate was only 48%.

In the studies of G Q Li et al (1982) artemether was used for the treatment of 116 confirmed cases of falciparum malaria (including 21 severely ill patients, one of whom died of severe cerebral malaria); 115 were clinically cured. At the total dose level of 240 mg (four cases) all patients showed recrudescences, while 10.4% recrudescences were observed among those having received total doses of 480, 600 or 640 mg over three or five days. Fever subsidence and initial parasite clearance times were similar under all dose regimens with means of 30.1 ± 21.6 hours and 60.6 ± 16.6 hours respectively.

In general initial parasite clearance and fever subsidence are fast following treatment with all formulations of Qinghaosu, artemether and sodium artesunate, but the rate of recrudescences is significantly high. Since this is obviously a dose- and probably also a time-dependent phenomenon it should be possible to arrive at more effective dose regimens through well-planned clinical studies.

The rapid action of Qinghaosu (i.m. or orally) has also been shown by Jiang et al (1982), who compared this drug with mefloquine and quinine for the treatment of falciparum malaria in adults. The mean fever clearance times were 30.6 hours for mefloquine given orally (1.0 g), 42.2 hours for quinine i.m. (0.5 g three times daily for three days), 33.7 hours for Qinghaosu water suspension i.m. (0.6 g first day, 0.3 g second day, 0.3 g third day), and 22.3 hours for Qinghaosu given orally (1.0 g first day, 1.0 g second day). The mean parasite clearance time was 103 hours with mefloquine, 104 hours with quinine, 79 hours with Qinghaosu i.m, and 68 hours with Qinghaosu given orally.

The effect of Qinghaosu is clearly dose-dependent also in vivax malaria, but independent of the drug formulation used, such as tablets, oil solution, oil suspension or water suspension. Short-term cure rates of 90% and more were obtained with all four formulations, approaching the efficacy of chloroquine, yet with a significantly shorter mean time required for parasite clearance, i.e. 31.5 hours as compared to 50.8 hours under chloroquine (CCRG 1982e).

No toxic effects were reported in any of the above-mentioned treatment groups. The limited range of biochemical parameters assessed, ECG findings and renal function tests showed normal values or improvement under treatment with Qinghaosu, artemether or sodium artesunate. However, pain occurred at the site of the i.m. injection of the aqueous suspension of Qinghaosu. Similarly, the oil solution, which required the injection of a considerable volume due to the poor solubility of the drug, produced tenderness and nodules at the injection site (CCRG 1982e).

Qinghaosu has also been formulated in suppositories which were used in the treatment of 100 patients, aged 1–16 years, who suffered from falciparum malaria (G Q Li et al 1985). The studies were carried out in Hainan Island. The patients received a total dose of 2800 mg Qinghaosu: 600 mg twice daily on the first day, and 400 mg twice daily on the second and third day. Fever subsided within 20.7 ± 11.8 hours; parasites cleared within 53.2 ± 15.6 hours. Parasite densities (initially 3120–216 500/μl with a mean

of 32 345/μl) decreased to 5% of their original level within 24 hours. There were 46.5% recrudescences. Local side effects were observed in 12% of the cases, but they were mild and subsided spontaneously within a few hours. There were no marked changes in the haematological parameters or in hepatic, cardiovascular and kidney functions.

Qinghaosu, artemether and sodium artesunate were also used in the treatment of cerebral malaria in Hainan Island and Yunnan Province (G Li et al 1982). Qinghaosu was given to 106 patients, 22 of whom suffered from extremely severe manifestations of cerebral malaria. Nine patients died. Fever subsided and parasites cleared more slowly than in uncomplicated cases of falciparum malaria; this delay was more marked when Qinghaosu was administered i.m. as a water suspension, as compared to water suspension given by nasogastric tube or the i.m. injection of a Qinghaosu solution in oil. Intramuscular injection of artemether in oil solution and intravenous injection of sodium artesunate in aqueous solution were used in 17 cases each. One patient of the former group and two of the latter group died. Fever and parasite clearance times in both groups were similar to Qinghaosu, but coma cleared fastest after sodium artesunate, with a mean of 12 hours after the start of treatment. The mean time (all formulations) required for fever subsidence was 38 hours, parasites cleared on average within 59 hours and recovery from coma took 23 hours. The water suspension of Qinghaosu (i.m.) appeared to have the relatively slowest effect, sodium artesunate the fastest. Considering that 21% of the patients were suffering from extremely severe cerebral malaria, the overall case fatality rate of 8.6% is quite low and well below the average recorded with other forms of treatment in this part of the world.

Considering the relatively high recrudescence rate following treatment with Qinghaosu, Cai et al (1981) treated 141 adults suffering from symptomatic falciparum malaria with a variety of drug regimens using: (1) 500 mg Qinghaosu i.m. plus 1000 sulfadimethoxine, 70 mg pyrimethamine and 30 mg primaquine per os; or (2) chloroquine total dose of 25 mg (base)/kg over three days per os; or (3) 500 mg Qinghaosu i.m. for two days; or (4) 1000 mg sulfadimethoxine, 70 mg pyrimethamine and 30 mg primaquine (base) per os. The cure rates were 100% in the first group (with no recrudescence during a 60-day follow-up) and 41%, 90% and 67% in groups 2, 3 and 4 respectively.

In another study, G Q Li et al (1984) compared (1) mefloquine plus sulfadoxine/pyrimethamine, (2) mefloquine plus Qinghaosu, (3) Qinghaosu and (4) mefloquine plus sulfadoxine/pyrimethamine plus Qinghaosu, in a total of 80 patients with falciparum malaria. Fever clearance was fastest under combination (4), while parasite clearance was fastest under Qinghaosu alone (3). No recrudescences were seen in groups 1, 2 and 4, but 41% recrudescences occurred in group 3. Since fever and parasite clearance under mefloquine and Qinghaosu (2) took longer than under Qinghaosu alone (3), Peto et al (1985) suggested the possibility of antagonism between Qinghaosu and mefloquine. This is in marked contrast to the findings in the *P. berghei* (N strain)/mouse system where both drugs exhibited synergism (Chawira et al 1984). In the latter system antagonism was observed between Qinghaosu and pyrimethamine, sulfadoxine and sulfadoxine/pyrimethamine.

However, in the clinical studies of G Q Li et al 1984), the quadruple combination (mefloquine, sulfadoxine, pyrimethamine and Qinghaosu) produced the fastest defervescence and also cleared parasitaemia more rapidly than mefloquine plus Qinghaosu. It may therefore be too early for drawing conclusions from limited clinical studies, but it is probably also too early to embark on clinical combination studies with Qinghaosu in the absence of exhaustive preclinical, toxicological and pharmacological observations.

Pharmacokinetics and assay methods

For experimental studies in vitro and in animals ^{3}H-labelled Qinghaosu and artemether have been employed (CCRG 1982f), but this label is not sufficiently stable. It would be preferable to have a ^{14}C label in a ring position. Considering the origin of Qinghaosu, the production of the ^{14}C-labelled drug would pose major technical problems, but the total synthesis of the compound by Schmid & Hofheinz (1983) has improved the prospects for approaches to the preparation of such a labelled

substance which would be very useful in the context of pharmacokinetic and pharmacodynamic studies.

A thin layer chromatographic (TLC) method has been developed for Qinghaosu with a sensitivity of 0.06 μg and a rate of recovery from tissue of 86%. With artemether the sensitivity is 0.1 μg and the rate of recovery 83%. Since sodium artesunate is rapidly transformed in vivo to dihydroqinghaosu, it is the latter that is being measured using TLC, producing a rather low sensitivity of 0.5 μg (CCRG 1982f).

More recently highly sensitive methods of nuclear magnetic resonance (NMR) spectrometry have been developed (Y Li et al 1983; Wang & Yin 1984). NMR and X-ray determination have also been used for determining the absolute configuration of Qinghaosu and its analogues (Luo et al 1984). Studies in mice and rats show that Qinghaosu in aqueous suspension is rapidly absorbed after oral administration, peak concentrations in blood being reached within one hour. Concentrations were highest in liver, brain, blood, plasma, lung, kidney, muscle, heart and spleen. In pregnant mice Qinghaosu was found to cross the placenta, appreciable drug levels being found in foetal liver, heart and intestinal tissue. The intraerythrocytic concentrations are lower than the plasma levels; a ratio of 1:4 having been observed 30 minutes after drug administration in mice; 48 hours later the ratio was 1:8. Whole body autoradiography in mice showed very high radioactivity in the gall-bladder and in the urinary bladder. The distribution pattern after the oral administration of ^{3}H-dihydroqinghaosu and the i.m. injection of ^{3}H-artemether was similar to that seen with Qinghaosu. However, intraerythrocytic and plasma concentrations of ^{3}H-artemether were roughly equal. Since these studies were made with ^{3}H-labelled Qinghaosu, they do not reflect the presence of active drug. In fact, it is probable that most of the activity found later than one hour after drug administration stems from inactive metabolites. Artemether binds to a significant extent with plasma protein: 58% in mice, 61% in rhesus monkeys and 77% in humans (CCRG 1982f). Binding of the parent compound Qinghaosu to human plasma protein is less (65%). It is least marked with the active metabolite dihydroqinghaosu (44%) (W H Li et al 1982).

Sodium artesunate given intravenously to rats is rapidly transformed into dihydroqinghaosu, the latter reaching highest concentrations in heart, muscle, lung, spleen, kidney, brain, blood and liver. Traces of the drug were found up to 80 minutes after administration.

Some 80% of radioactivity following oral administration of ^{3}H-Qinghaosu and ^{3}H-dihydroqinghaosu is excreted within 24 hours in faeces and urine (mouse). The elimination is slower after the intravenous administration of ^{3}H-artemether.

Following the intravenous administration of Qinghaosu and artemether to rats or rabbits, the drug plasma levels as opposed to the time curve appeared to fit two-compartment open models, the parameters of which are given in Table 51.5. The bioavailability of an i.m. injected aqueous suspension of Qinghaosu in rats was 50%, while that of an oily suspension of artemether in rabbits was 37–50%. The plasma levels of sodium artesunate fitted a one-compartment open model with the following parameters: $t_{½}$ = 15.6 min; K = 0.0444/min; VD = 1.1 l/kg (CCRG 1982f).

Studies on the biotransformation of ^{3}H-Qinghaosu and ^{3}H-dihydroqinghaosu (Zhu et al 1980; CCRG 1982f) showed that most of the drug is metabolized within 30 minutes after i.v. injection. In vitro experimentation with rat tissue slices indicated a very high metabolic activity of liver tissue which metabolized 91.7% of the total available Qinghaosu within one hour. Lesser, yet significant, activity was also found with kidney and lung tissue. Four metabolites were found in the urine of patients

Table 51.5 Pharmacokinetics of Qinghaosu and artemether following i.v. administration; 2-compartment open model. (data from CCRG 1982f)

	Qinghaosu (rat)	Artemether (rabbit)
$t_{½}\alpha$	2.66 min	6.84 min
$t_{½}\beta$	30.13 min	39.60 min
K_{21}	0.057/min	0.032/min
K_{12}	0.112/min	0.029/min
K_{10}	0.105/min	0.072/min
V_C	0.90 l/kg	0.75 l/kg
V_B	4.1 l/kg	3.0 l/kg

treated with Qinghaosu. The metabolites have no antimalarial activity. Three of these metabolites were identified as dihydrodeoxyqinghaosu (Fig. 51.9), deoxyqinghaosu (Fig. 51.10) and 'crystal 7' (Fig. 51.11). The loss of the peroxide bridge obviously results in the loss of antimalarial activity.

In experiments with ^{14}C-labelled artemether (label on the ether C) in mice, 15% of the radioactivity was exhaled in the form of $^{14}CO_2$, indicating that demethylation occurs in vivo, a phenomenon that could be induced by phenobarbital.

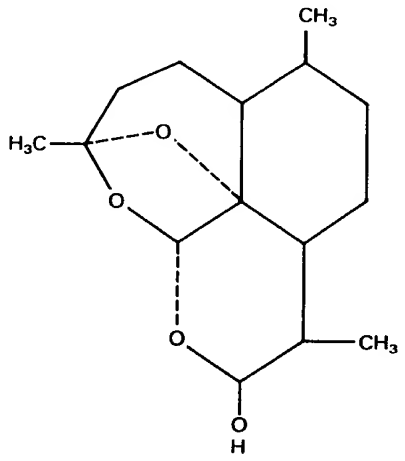


Fig. 51.9 Dihydrodeoxyqinghaosu

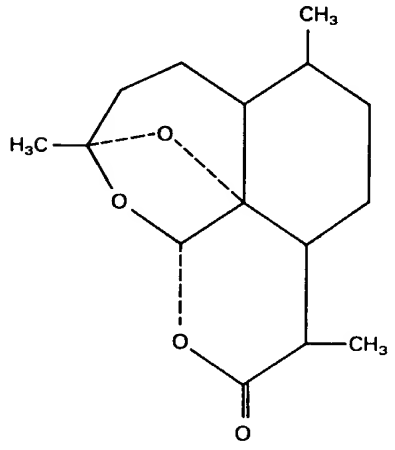


Fig. 51.10 Deoxyqinghaosu

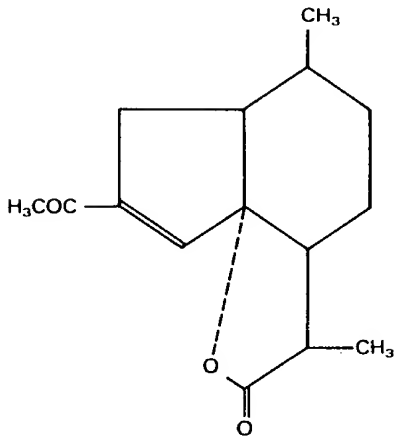


Fig. 51.11 Crystal 7

Mode of action

Please refer to the section on the mode of action of antimalarial drugs (p. 1578).

Prospects

Qinghaosu, artemether and sodium artesunate are effective against *P. falciparum* and *P. vivax*. Their mechanism of action appears to be different from that of all hitherto used antimalarial drugs. They are obviously subject to a marked first pass effect when given by the oral route. This reduces the practical feasibility of oral administration. Acceptable parenteral formulations can be obtained with artemether, sodium artesunate and artesunic acid. The latter two can be formulated as i.v. injectables which should be particularly useful for the emergency treatment of severe and complicated forms of falciparum malaria. In the context of primary health care, it would also be useful to have a safe and easy-to-use formulation for intramuscular injection. Moreover, preliminary investigations with suppositories indicate that rectal administration is feasible. At health service levels where staff are not habilitated to administer parenteral administration this may solve problems

mg/kg for chloroquine (base). Intramuscular administration yielded an ED_{50} of 5.0 mg/kg for pyronaridine and 30.9 mg/kg for chloroquine. In another test series mice were infected with *P. berghei* some four hours after the first oral doses of pyronaridine, chloroquine or mepacrine had been given. The mice were treated again on the two following days and blood smears were examined on the seventh day. With this schedule the ED_{50} was between 2.01 and 2.88 mg/kg for pyronaridine, 15.90 mg/kg for chloroquine, and 17.70 mg/kg for mepacrine when the chloroquine-sensitive isolate of *P. berghei* was used. In contrast, the ED_{50} with the chloroquine-resistant isolate of *P. berghei* was not significantly different for pyronaridine (0.99–1.10 times), but considerably higher for mepacrine (22 times) and exceeding a measurable range for chloroquine (>52 times).

Results in *Macaca mulatta* infected with *P. inui, P. cynomolgi* or *P. knowlesi* were ambiguous, probably on account of inadequate dose regimens.

Acute toxicity studies in mice yielded an LD_{50} of 1369 mg/kg after oral administration of pyronaridine and 251 mg/kg after i.m. administration. The corresponding values for chloroquine were 663 and 90 mg/kg respectively. Rabbits and dogs tolerated single i.m. doses of up to 40 mg pyronaridine/kg. Dogs given total oral doses of 120 mg and 240 mg pyronaridine within three days all vomited but showed no other serious side effects. The biochemical parameters stayed within normal limits. Toxic manifestations in dogs having received i.m. doses of over 40 mg pyronaridine/kg included clonus, heart block and raised SGPT. No such symptoms were seen at lower doses. No toxic symptoms or ECG changes were seen in four monkeys given 60 mg pyronaridine/kg twice a day on the first day and once a day on the next two days (total dose 240 mg/kg), but one of the animals showed a temporary SGPT rise from 20 to 107 units, with normalization within one week.

In studies of subacute toxicity, rats received pyronaridine at an oral dosage of 200 mg/kg or 40 mg/kg daily for 14 days. At the higher dosage diminished appetite and retarded growth were observed; 20% of the animals died within two weeks. At the lower dosage one out of 15 animals died, and the surviving rats showed no signs of toxicity. Rabbits given 10 mg pyronaridine/kg as an i.v. drip twice on the first day and once daily on the following days tolerated the drug well and without pathological changes of ECG and biochemical parameters. Similarly, dogs given oral doses of 12 and 24 mg/kg daily for 28 days showed no signs of toxicity and no abnormal ECG or biochemical findings.

In anaesthetized rabbits, dosed with 4 mg pyronaridine every two minutes through the severed carotid vein, the blood pressure dropped to 5.33 KPa when the cumulative dose had reached 40 mg/kg. ECG changes showed initially fused T and P waves, prolonged P-R and Q-T intervals and broadened QRS, followed by A-V block or sinus bradycardia, and premature ventricular beat. An autoventricular rhythm set in at an average cumulative dose of 65 mg/kg, followed by death. Similar studies in dogs yielded nearly identical ECG findings, but drastic decrease of blood pressure and death occurred at cumulative doses of 98 mg/kg and 116 mg/kg.

Mutagenicity tests with the *Salmonella typhimurium*/microsome system showed an induction of mutations without metabolic activation in strain TA 1537 at 100–1000 μg/plate. Reversion was dose-dependent and similar to chloroquine. There was no induction of mutation in strains TA 98, TA 100, TA 1535 and TA 1538 (Ni et al 1982a).

Teratogenicity tests in rats conducted with the equivalent of 8, 15, 30 or 33 times the clinical doses yielded no evidence of teratogenic effects, but the rate of foetal resorption was significantly increased (Ni et al 1982b).

Resistance to pyronaridine could be induced through increasing subcurative doses of the drug in the *P. berghei* model. Within 23 passages it reached a very high level with refractoriness to a dose of 2400 mg/kg. The virulence of the pyronaridine-resistant isolate was much reduced. The isolate showed also reduced sensitivity to mepacrine, 4-aminoquinolines and Qinghaosu. Without drug pressure the pyronaridine-resistant line reverted to pyronaridine sensitivity within five passages (Shao et al 1982).

Pharmacokinetic studies in rabbits indicate peak plasma concentrations of pyronaridine 15

minutes after i.m. injection. The drug concentrations in plasma, erythrocytes and tissues decreased rapidly within three hours. Pyronaridine had also been independently tested by the Walter Reed Army Institute of Research. It effected cures against a chloroquine-resistant strain of *P. berghei* in mice in a schizontocidal test at doses of 20–640 mg/kg, with toxicity observed at the highest dose. It was effective in a suppressive test against both chloroquine-sensitive and -resistant strains of *P. berghei*, although the SD_{90} values were slightly higher with the resistant strain. In the in vitro testing against *P. falciparum*, it showed similar activity against both the sensitive and resistant isolates (EC_{50} 1.26 and 2.09 ng/ml) respectively). Thus, this drug does not appear to have cross-resistance with chloroquine (Canfield, personal communication).

Clinical studies

After safety studies at the Institute of Parasitic Diseases, Shanghai, clinical trials were conducted in Hainan Island, where *P. falciparum* is predominantly chloroquine-resistant. More than 1000 cases of vivax and falciparum malaria were treated, including patients with cerebral malaria. Most cases were treated with a total oral dose of 24 mg pyronaridine/kg, with the first two doses of 6 mg/kg each being given on the first day, and the same doses once daily on the second and third day. For parenteral administration (i.m.), two doses of 3 mg/kg were given at a 6-hour interval. Doses for i.v. infusion were adjusted accordingly, up to a total of 6 mg/kg. The recrudescence rate among the 342 cases of falciparum malaria which were followed for 30 days after treatment was 8.8%. It was highest in the patients having received oral treatment (14%) and lowest (0%) in those treated by i.v. infusion. Fever clearance was fastest in the parenterally treated groups (average 27 hours), with little difference between i.m. and i.v. treated patients, as compared to 44 hours in patients having received the drug orally. Little difference was observed in the parasite clearance times (average 52 hours). Clearance of fever and parasitaemia was relatively faster in vivax malaria (22 hours and 45 hours respectively).

Patients with cerebral malaria and chloroquine-resistant *P. falciparum* responded well to treatment with pyronaridine. Side effects following oral treatment included nausea, diarrhoea and slight abdominal pain, vomiting, palpitations, headache and allergic skin rash. However, most of these symptoms may be directly or indirectly related to malaria rather than to the drug. Less side effects were observed following parenteral treatment, with an incidence of slightly over 3%; these included loss of appetite, nausea, abdominal pain, diarrhoea, palpitations, and one case of allergic skin rash whose exanthema disappeared within one day after antiallergic treatment. Irritation at the site of intramuscular injection was recorded in six out of 249 cases, but this resolved spontaneously within a few days without necrosis or the formation of abscesses. ECG, haematological findings, blood pressure, respiration and urinalysis showed no pathological changes which could be ascribed to the treatment with pyronaridine.

Prospects

Pyronaridine is a promising antimalarial compound which is effective also against chloroquine-resistant plasmodia. However, patterns of cross-resistance with other known antimalarials and the ease with which resistance to the pyronaridine series arises should be further determined. The preclinical data are also still rudimentary, and little is known about the pharmacokinetics of the drug and thus about the possibilities of optimizing its use through appropriate galenic formulation. These lacunae would need to be filled before the implementation of meaningful clinical trials which would first have to assess tolerability and bioavailability, and then determine the most effective dosage regimen. The observation of skin rash may require special attention. From the studies of Zheng et al (1982) it appears that pyronaridine and some of its recently synthesized analogues possess causal prophylactic activity in rodent models (*P. berghei* and *P. yoelii*), with an efficacy superior to primaquine. This merits further investigation in view of the need for well-tolerated tissue schizontocides.

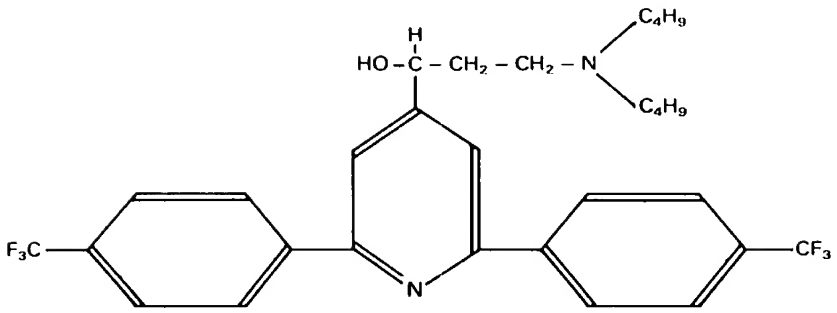


Fig. 51.13 WR 172 435

Enpiroline (WR 180 409)

Markovac et al (1972) synthesized a series of 21 trifluoromethyl-substituted α-alkylaminomethyl-2,6-bis(aryl)-4-pyridinemethanols, after compounds bearing halogen or methoxy-substituents on the aryl rings had shown antimalarial activity in the *P. berghei*/mouse screen (Blumbergs et al 1972). Of the 21 trifluoromethyl-substituted compounds, 11 were curative at 40 mg/kg and two of these at only 10 mg/kg. Later on, three pairs of racemic α-(2-piperidyl)-2,6-disubstituted 4-pyridinemethanols were synthesized (LaMontagne et al 1974) and found to possess curative antimalarial activity in the *P. berghei*/mouse screen, four being uniformly curative at 40 mg/kg. The investigations were conducted within the framework of the chemotherapeutic research programme of the Walter Reed Army Institute of Research which pursued also the further development of these compounds, 10 of which were studied in the *P. falciparum*/*Aotus trivirgatus* system (Schmidt et al 1978c). Three of the compounds were approximately two to three times more active than chloroquine against chloroquine-sensitive *P. falciparum*. The required doses of these three 4-pyridinemethanols proved to be independent of resistance to chloroquine, quinine and pyrimethamine. These three compounds are: WR 172 435 [3-di-n-butylamino-1-(2,6-bis(4-trifluoromethylphenyl)4-pyridylpropanol-methanesulfonate], WR 180 409 [dl-threo-α(2-piperidyl)-2-trifluoromethyl-6-(4-trifluoromethylphenyl)-4-pyridinemethanol phosphate] and WR 180 117 (erythro-isomer of WR 180 409). The structural formulae of WR 172 435 and WR 180 409 are given in Figures 51.13 & 51.14. Schmidt et al (1978c) found WR 172 435 to be slightly more active than WR 180 409. WR 180 117 was not followed up further since it showed no therapeutic advantage over its stereoisomer WR 180 409 in the advanced screen. Oral administration of identical doses of WR 172 435 or WR 180 409 over three and seven days was equally effective, and slightly superior to

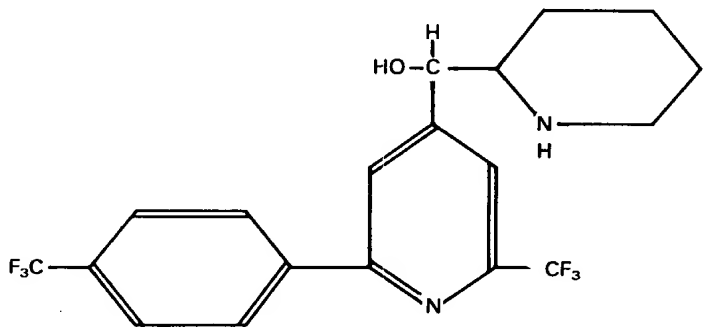


Fig. 51.14 WR 180 409

single-dose administration. The approximate CD_{90} of WR 172 435 against the Vietnam Smith isolate of *P. falciparum* was 26 mg/kg for the single dose, 15 mg/kg for the three-dose regimen and 16 mg/kg for the seven-dose regimen. The corresponding values for WR 180 409 were 35, 30 and 24 mg/kg. *P. vivax*, the Vietnam Palo Alto isolate, proved to be more sensitive with an approximate CD_{90} of 5 mg/kg for the single dose, 6 mg/kg for the three-day regimen and 7 mg/kg for the seven-day regimen with WR 172 435. The corresponding values for WR 180 409 were 14, 18 and 16 mg/kg respectively. The therapeutic indices were at least four to eight times those of chloroquine against infections with chloroquine-sensitive *P. falciparum*. Both compounds controlled parasitaemia faster than any of the standard drugs or other synthetic candidate compounds. WR 180 409, formulated as the phosphate, can also be given by the intravenous route. Both compounds have blood schizontocidal activity against *P. falciparum* and *P. vivax*; they are ineffective against exoerythrocytic forms.

WR 172 435 is less toxic than WR 180 409; however, both compounds have acceptable levels of toxicity in mice, rats and dogs (Schmidt et al 1978c). They lack phototoxicity and specific organ toxicity. WR 172 435 showed a lesser acute toxicity than mefloquine, after both oral administration (LD_{50} 2754 mg/kg versus 880 mg/kg) and i.p. administration (LD_{50} 254 mg/kg versus 130 mg/kg). Both WR 172 435 and WR 180 409 showed also a lower subacute toxicity than mefloquine in rats and dogs (Canfield 1980). WR 172 435 and WR 180 409 (enpiroline) were selected for clinical trials, but studies on the former compound were discontinued as it caused (reversible) leukocytosis in healthy male volunteers (Cosgriff et al 1984).

Toxicological and pharmacokinetic studies with WR 180 409 in man showed good tolerability of single doses up to 1 g. Larger single doses provoked nausea, vomiting and dizziness (Bruce-Chwatt et al 1981). The compound seemed to be well absorbed and distributed in the tissues, the majority being excreted via the bile in the faeces in the course of 20 days. After the administration of single oral doses of 750 mg in healthy adult males, the mean peak concentration in plasma was 0.9×10^{-6} mol/l, with a mean time to peak (tp) of 13.2 hours. The mean half-life was estimated to be six days (Cosgriff et al 1984).

In a clinical phase II dose-finding study enpiroline (WR 180 409) was administered to 22 non-immune adult males with induced infections of the chloroquine-, quinine- and pyrimethamine-resistant Vietnam Smith *P. falciparum* isolate (Cosgriff et al 1984). Oral treatment with three doses of 500 mg every 12 hours, or with 500 mg, 500 mg and 250 mg, or 500 mg, 250 mg and 250 mg at 12-hourly intervals was uniformly curative. Similar results were obtained following treatment with 500 mg followed 12 hours later by another dose of 250 mg (12 patients). This represents a minimum total curative dose level of 10.2 ± 1.3 mg/kg body weight. A single dose of 500 mg produced defervescence and initial parasite clearance which was followed by recrudescence. The mean fever clearance time was 67 ± 32 hours, the mean parasite clearance time 48 ± 10 hours. The drug was generally well tolerated; apart from nausea, vomiting and diarrhoea which are also associated with falciparum malaria, there were no other side effects.

There might have been some initial reluctance in undertaking clinical trials because of the expectation of cross-resistance between all members of the aminoalcohol group, i.e. 4-quinolinemethanols, 9-phenanthrenemethanols and 4-pyridinemethanols. This assumption seemed to be less likely on the basis of the relationships between 4-aminoquinolines and 9-phenanthrenemethanols. (Cosgriff et al 1982). The full activity of WR 180 409 against the multidrug-resistant Vietnam Smith isolate of *P. falciparum* augured well for this compound, all the more so as Schmidt et al (1978c) did not find at any stage of their investigations any signs of emerging resistance to the 4-pyridinemethanols.

However, subsequent studies conducted by the Walter Reed Army Institute of Research indicate that enpiroline has no distinct advantages over either mefloquine or halofantrine.

CANDIDATE ANTIMALARIALS IN AN ADVANCED PRECLINICAL STATE

This is a heterogenous group of compounds which includes 4-aminoquinolines, 8-aminoquinolines, 4-quinolinemethanols, quinolones, naphtho-

quinones, quinazolines and dihydrotriazines. Most of the compounds were identified and investigated in the drug development programme of the Walter Reed Army Institute of Research.

4-aminoquinolines and Mannich bases

Chloroquine and amodiaquine, the best known representatives of this class of blood schizontocides, were universally useful until the occurrence of resistance restricted their efficacy against *P. falciparum* in wide areas of eastern Asia, South America and Africa. Although both compounds were initially considered equivalent with regard to efficacy and type of activity, it became clear later that amodiaquine was more effective in vitro than chloroquine (Spencer et al 1983). This difference is also reflected in a higher clinical activity of amodiaquine against chloroquine-resistant falciparum malaria (Hall et al 1975b; Watkins et al 1984; Spencer et al 1984). Nevertheless the therapeutic advantage may not be sufficiently great or reliable to warrant its use in areas with high degrees of chloroquine resistance (Pinichpongse et al 1982; Campbell et al 1983) unless it is combined with another, effective drug. In such circumstances Noeypatimanondh et al (1983) obtained highly satisfactory results with amodiaquine and tetracycline for the treatment of uncomplicated falciparum malaria in Thailand. In the virtual absence of RII and RIII resistance, amodiaquine made a major clinical and parasitological impact which was consolidated by tetracycline.

The tacitly accepted dogma of cross-resistance between all members of the 4-aminoquinoline class is no longer tenable, especially after the investigation of some new compounds. In this connection, it would have been of interest to assess the activity and cross-resistance pattern of some of the older 4-aminoquinolines such as 7-chloro-4-(3′-pyrrolidyl-4′-hydroxyanilino)quinoline (amopyroquine) and 7-chloro-4-[3′,5′-bis(diethylaminomethyl)-4′-hydroxyanilino]quinoline (cycloquine). These compounds never became competitive with chloroquine on account of their higher price.

New 4-aminoquinoline type compounds have been developed by the expansion of the ring structure by adding a benzene ring to the quinoline. Such a compound is benzo(g)-4-(diethylamino-

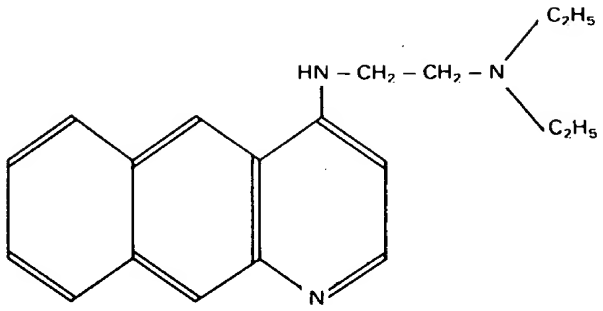


Fig. 51.15 Dabechin (G 800)

ethylamino)quinoline (G 800, Dabechin, Fig. 51.15) which was synthesized in the Soviet Union (Bechli et al 1977) and nearly equals chloroquine in blood schizontocidal activity in the *P. berghei*/mouse model. However, its half-life in mice is longer than that of chloroquine (Soprunova et al 1977) and the tolerance is better. Nevertheless, cross-resistance between G 800 and chloroquine offers apparently little prospect for this compound and its 4-diethylaminopropylamino-analogue (G 734).

The synthesis of long-acting analogues of amodiaquine, hydroxychloroquine and oxychloroquine was pursued by Elslager et al (1969d). The amide congeners proved to have no significant repository antimalarial activity. However, various o-cresol, alkanol, ethanol and propanol esters, formulated mainly as pamoates, showed slight to moderate activity in protecting mice against challenge with *P. berghei* for two to four weeks, when given as a single i.m. dose of 400 mg/kg. This group was not followed up any further since other candidate compounds showed more promising repository activity, and chloroquine resistance had started to compromise the prospects of 4-aminoquinolines.

Earlier studies with 7-chloroquinoline, substituted in the 4-position by piperazines, produced evidence of some antimalarial activity of the 4-aminoquinoline type. A symmetric compound linking two molecules of 7-chloro-4-(1-piperazinyl)-quinoline through a propyl group (piperaquine, Fig. 51.16) possesses blood schizontocidal activity similar to that of chloroquine.

There was, for a while, little interest in this compound and its analogues (12278 RP, 12494 RP, 14153 RP) since field trials had failed to show

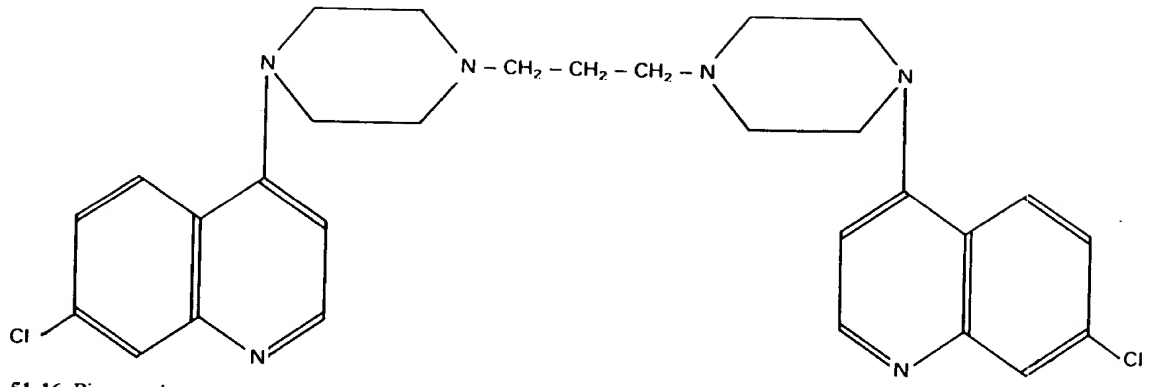


Fig. 51.16 Piperaquine

major advantages over chloroquine (WHO 1973). Later experimental studies with piperaquine in the *P. berghei*/mouse system (Zhu et al 1982) suggest that this compound is more effective, longer acting and less toxic than chloroquine. Although the field studies of a Chinese group in Hainan Island indicated that piperaquine had a high suppressive and therapeutic activity (Chen et al 1982), no conclusions can be drawn from this investigation in the absence of a comparison with chloroquine. Moreover, these studies were conducted in 1972, when chloroquine resistance had probably not yet occurred in this area.

Hydroxypiperaquine (Fig. 51.17), an analogue of piperaquine, was synthesized in China (Y T Li et al 1982). Acute, subacute and chronic toxicity in mice, dogs, rabbits and monkeys is less than that of chloroquine. It does not apparently exert mutagenic, teratogenic or embryotoxic activity. In *P. berghei*-infected mice and in *P. cynomolgi*-infected monkeys treated with this compound the onset of parasite clearance appears to be slower than with chloroquine, but there is no marked difference in the absolute clearance time. However, recrudescences were more frequent in the monkeys treated with hydroxypiperaquine than in those having received chloroquine (Y T Li et al 1984). Chloroquine-resistant *P. berghei* was almost as sensitive to hydroxypiperaquine as the chloroquine-sensitive isolate (efficacy ratio 1.38:1.00 as compared to chloroquine 62:1). In clinical trials in Yunnan Province, an area with a moderate frequency of chloroquine-resistant falciparum malaria, hydroxypiperaquine treatment led in all 93 cases to fever clearance within 72 hours (mean 28.2 hours), and parasite clearance within 96 hours (mean 50.2

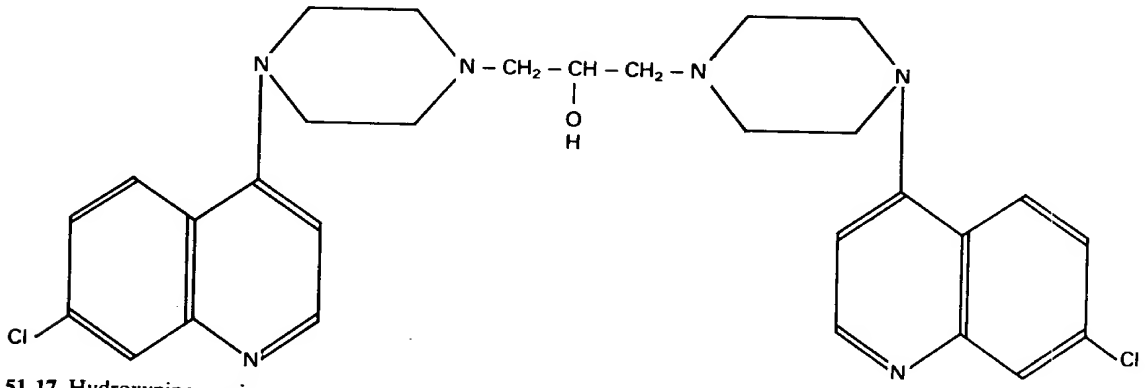


Fig. 51.17 Hydroxypiperaquine

hours). Only one of the 60 cases observed for four weeks produced a recrudescence. The drug was given at a total adult dose of 1.5 g base, over three days (0.6 g, 0.6 g, 0.3 g).

Chloroquine, in the same dose regimen, failed to produce parasite clearance within seven days (RII, RIII) in seven out of 28 cases, follow-up of 19 others showed five recrudescences (RI). Parasite and fever clearance among the S/RI cases were slower than with hydroxypiperaquine (67.8 and 42.0 hours respectively), and hydroxypiperaquine was better tolerated than chloroquine (Y T Li et al 1981a).

In Hainan Island, an area with a high frequency and degree of chloroquine resistance, 158 cases of acute falciparum malaria were treated with chloroquine (1.5 g base over three days as adult dose); there were 45 RII and 23 RIII responses. These latter patients were given hydroxypiperaquine at the total dose of 1.5 g base over three days; fever and parasites cleared rapidly (mean clearance time 37.2 and 52.4 hours respectively). The patients were discharged from hospital after treatment; there were subsequently three late recrudescences (days 21 and 28) among 64 patients followed up for four weeks (Y T Li et al 1981b). These cases could also have been new infections.

Hydroxypiperaquine is also effective as a blood schizontocidal drug against *P. vivax*. Given at doses of 0.9–1.2 g base over two days it produced parasite clearance in all 718 cases with a mean clearance time of 41.3 hours; chloroquine, at a dose of 1.2 g base over two days, also led to parasite clearance in all 356 patients so treated, with a mean parasite clearance time of 37.9 hours (personal communication, Laboratory for Antimalarial Drug Research, Second Military Medical College, Shanghai).

Tripiperaquine, i.e. 1,4-bis[1-(7-chloro-4 quinoline)-4-ethyl-piperazinyl] piperazine, or M1020, was synthesized in China (Fig. 51.18) and studied for its activity and toxicity in mice and monkeys. In *P. berghei*-infected mice the ED_{50} of tripiperaquine was lower than that of chloroquine; the suppressive blood schizontocidal activity lasted longer than with chloroquine both in *P. berghei*-infected mice and *P. cynomolgi*-infected rhesus monkeys. However, against established blood infections with *P. cynomolgi* and *P. inui* blood schizontocidal activity was not complete, but tripiperaquine became curative when associated with modest doses of sulfadoxine and pyrimethamine. The toxicity of tripiperaquine is less than that of chloroquine.

Clinical studies were conducted in patients with vivax and falciparum malaria, using tablets containing 100 mg tripiperaquine, 25 mg sulfadoxine and 2.5 mg pyrimethamine (Laboratory of Malaria Research, Shanghai Institute of Parasitic Diseases 1975). Adults were given a first dose of six tablets, followed six to eight hours later by four tablets amounting to a total dose of 1000 mg tripiperaquine, 250 mg sulfadoxine and 25 mg pyrimethamine. Children received a lesser dose according to age. All cases of vivax malaria responded with fever and parasite clearance (19.1 hours and 35.0 hours respectively as compared to

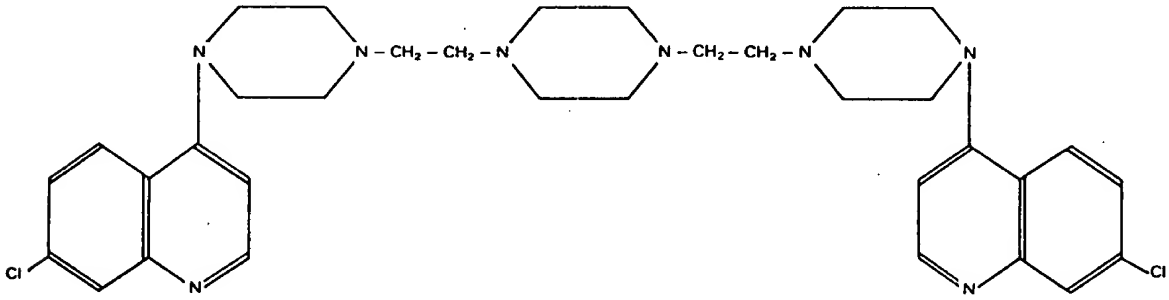


Fig. 51.18 Tripiperaquine (M 1020)

32.7 hours and 48.1 hours in the chloroquine-treated group). Five among the 31 cases followed relapsed on days 23, 28, 30 (two cases) and 31 respectively. All 29 cases of falciparum malaria showed a clearance of fever and parasites. Two of the 18 patients followed up for one month showed relapses of *P. vivax*, but there were no recrudescences of *P. falciparum*.

A structurally related compound, dichlorquinazine (Fig. 51.19), synthesized by Rhone-Poulenc, showed no cross-resistance with chloroquine in *P. falciparum* in vitro (Le Bras et al 1983) and seems worth further evaluation.

Interest in Mannich bases was stimulated when structural modifications of an earlier orthocresol derivative (SN 7744) yielded a biphenyl compound with remarkably high antimalarial activity, namely WR 194 965 [2-(t-butyl-aminomethyl)-4-t-butyl-6-(4-chlorophenyl)-phenol] (Osdene et al 1967; Schmidt & Crosby 1978) (Fig. 51.20). Although this compound was less active than chloroquine and amodiaquine in the rodent screen, it was decided to follow it up on account of its low toxicity. In the *P. cynomolgi*/rhesus and *P. falciparum*/*Aotus* models it proved to be approximately ten times as effective as amodiaquine (Sweeney et al 1983). WR 194 965 has undergone further preclinical studies, and clinical observations in volunteers indicate good tolerability.

Following the lead provided by SN 7744 and WR 194 965, a large series of Mannich bases was synthesized in which the 4-tertiary butyl group was substituted by a 7-chloro-4-quinolinylamino group. This yielded hybrids between the biphenyls and amodiaquine. Many of these hybrids showed significant antimalarial activity. WR 228 258 (Fig. 51.21) is one of the most active compounds (Pick 1982; Sweeney et al 1983). In the *P. berghei*/mouse screen it is more active than WR 194 965 or amodiaquine. Both WR 194 965 and WR

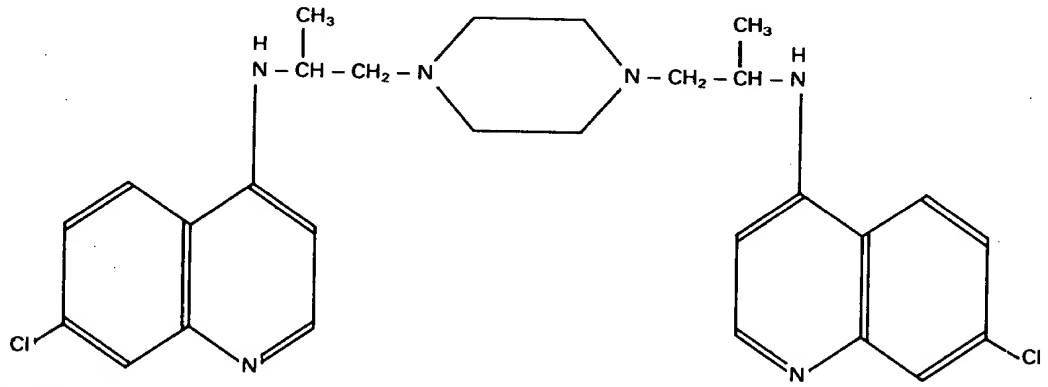


Fig. 51.19 Dichlorquinazine

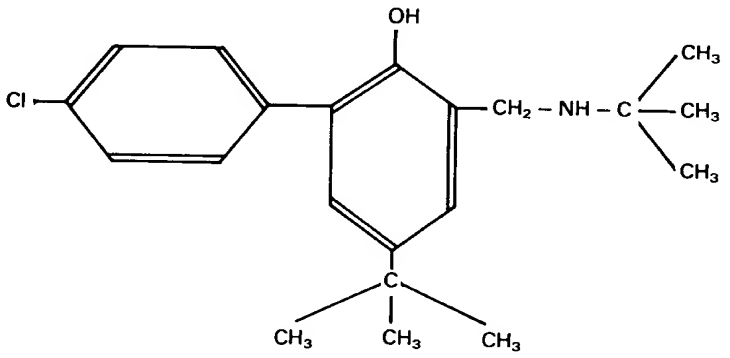


Fig. 51.20 WR 194 965

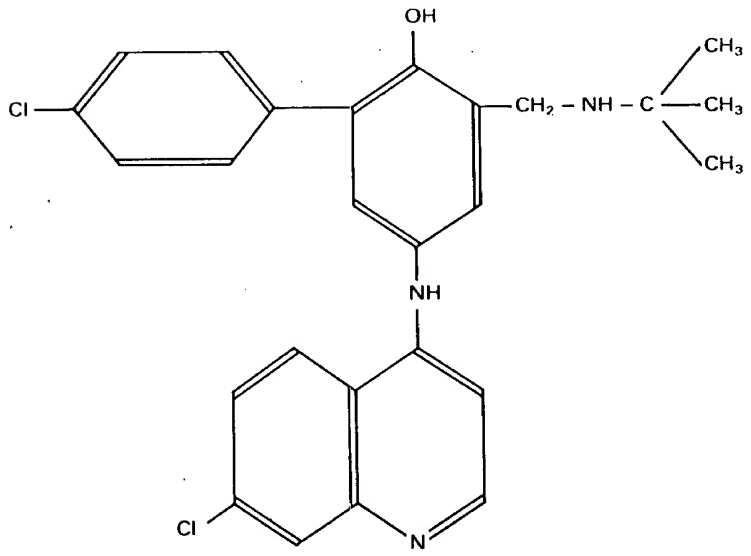


Fig. 51.21 WR 228 258

228 258 show a slightly reduced activity against the mefloquine-resistant N/1100 line of *P. berghei* (Peters & Robinson 1984b). The minimum curative dose in the *P. cynomolgi*/rhesus monkey system is < 1 mg/kg body weight. The CD_{50} in *Aotus* infected with the chloroquine- and pyrimethamine-sensitive Uganda Palo Alto isolate of *P. falciparum* is 1 mg/kg body weight. It is also 1 mg/kg in owl monkeys infected with the multiresistant Smith isolate of *P. falciparum*, and not significantly different from the CD_{50} observed with the chloroquine-resistant Oak Knoll isolate. These findings were supplemented by in vitro studies with *P. falciparum* isolates, indicating an absence of cross-resistance with chloroquine (Peters et al 1984b). WR 228 258 has a very long biological half-life, making this hybrid compound one of the most interesting and promising current leads in malaria chemotherapy.

Another approach to complex compounds was made by Go et al (1981), who hybridized molecules of amodiaquine and diethylcarbamazine in an attempt to obtain substances with antimalarial and antifilarial activity. One of the compounds showed twice the activity of amodiaquine in the *P. berghei*/mouse screen, and approximately half the toxicity. There was no antifilarial activity in *L. carinii*/*Mastomys natalensis*.

Incidentally, the concept of hybridizing active principles in the form of Mannich bases is not a new one. Nabih (1972) obtained a compound with marked antimalarial activity by condensing 2-diethylaminomethyl-4-amino-5,6,7,8-tetrahydro-l-naphthol and 4,7-dichloroquinoline.

8-aminoquinolines

This group of drugs includes the first synthetic antimalarial drug, pamaquine. This drug was used as a tissue schizontocidal compound until after World War II when less toxic 8-aminoquinolines became available as a result of the Survey of Antimalarial Drugs and the subsequent studies under the aegis of the US Public Health Service (Sweeney et al 1983). Four compounds were found to be superior to pamaquine, namely pentaquine, isopentaquine, 6-methoxy-aminobutylaminoquinoline (SN 3883) and primaquine. These four compounds were undergoing clinical trials when the Korean War and the need for large-scale radical treatment of returning US troops precipitated the selection of one compound, i.e. primaquine, before an exhaustive comparison with the other candidates was possible. However, the need for adminis-

tering primaquine in radical treatment of vivax and ovale malaria for 14 days, at a daily adult dose of 0.25 mg/kg, and the relatively high toxicity of the compound, especially in glucose-6-phosphate dehydrogenase (G6PD) deficient subjects, led to renewed interest in better 8-aminoquinolines. The Walter Reed Army Institute of Research therefore investigated a large number of new 8-aminoquinolines. A number of primary and secondary aminoheptyl or aminooctyl derivatives of 8-aminoquinolines proved to have blood schizontocidal activity in the Rane test, but their activity was not superior to that of primaquine and the toxicity was obviously not diminished (Wetter & De Witt Blanton 1974). Eight of these compounds were also tested for causal prophylactic activity in *P. yoelii yoelii*-infected mice, but none was as effective as primaquine. One of the compounds, an aminooctyl derivative, was studied in the *P. cynomolgi/Macaca mulatta* screen, but it was inactive at 10 mg/kg, while primaquine was effective at 1 mg/kg. In spite of these disappointing results, some Chinese workers showed interest in this group of compounds (Shao & Ding 1982), but it seems that the ^{3}H-labelled aminooctyle primaquine was to be used for pharmacokinetic studies in rats (Pan et al 1982) rather than as a viable basis for further development of this specific group. In this connection it is interesting to note that a short-chain 5-alcoxy derivative (5-propoxyprimaquine) has a four times higher radical curative activity and the same mouse toxicity as primaquine (Sweeney et al 1983).

In contrast, the 5-phenoxy derivatives of primaquine appear to hold considerable interest for future development. Chen et al (1977) synthesized numerous compounds of this class, some of which proved to be more active and less toxic than primaquine in the blood schizontocidal mouse screen. 5-(4-chloro)-phenoxyprimaquine and 5-(4-fluoro)-phenoxyprimaquine produced also radical cure in the *P. cynomolgi/Macaca mulatta* model, the latter compound being superior to primaquine. Replacement of the lipophilic halogen group by an acetamido group abolished the activity. Chinese scientists also became interested in this group of compounds, producing a number of 5-phenoxy-6-methoxy-8-(4-phthalimido-l-methylbutylamino) quinolines and 5-phenoxy-6-methoxy-8-(5-phthalimidoamylamino) quinolines some of which exhibited antimalarial activity, though inferior to that of primaquine (Zheng et al 1981). They studied also the 5-(4-fluoro)-phenoxyprimaquine in *P. cynomolgi*-infected rhesus monkeys and found it to be less active than primaquine. In mice this compound was approximately four to five times less effective and 20 times less toxic than primaquine. In later investigations of an expanded series of 5-phenoxy derivatives of primaquine (Sweeney et al 1983), two compounds showed primaquine activity indices higher than 1.0; the first, 5-(3-trifluoromethyl)-phenoxyprimaquine, had an index of 2, the second, 5-(3-fluoromethyl-4-fluoro)-phenoxyprimaquine, an index of 5. The minimum toxic dose (MTD), i.e. the minimum dose of drug causing at least 20% of drug deaths in the blood schizontocidal *P. berghei*/mouse screen, is higher than 640 mg/kg with both compounds, as compared to 160 mg/kg for primaquine. 2- or 4-methyl substitution of the 5-phenoxyprimaquines led to a significant increase of their radical curative efficacy, except for 5-(3-trifluoromethyl-4-fluoro)-phenoxyprimaquine, where 4-methyl substitution produced a less active compound. The MTD of all highly active 5-phenoxyprimaquines and their 2- or 4-methyl substituted analogues remained at a level of 640 mg/kg.

There are now four reported compounds with a primaquine activity index of 5 (i.e. these compounds have five times the radical curative activity of primaquine) and an MTD of 640 mg/kg in the mouse. These are 4-methyl-5-(3-trifluoromethyl)-phenoxyprimaquine (Fig.51.22; WR 225 448), 4-methyl-5-(2,4-dichloro)-phenoxyprimaquine (Fig. 51.23; WR 233 195), 4-methyl-5-(3,4-dichloro)-phenoxyprimaquine (Fig. 51.24; WR 233 078), probably the most active of this group, and 5-(3-trifluoromethyl-4-fluoro)-phenoxyprimaquine (Fig. 51.25; WR 242 471). These compounds have also a much higher blood schizontocidal activity than primaquine. This rendered studies of an eventual causal prophylactic activity difficult, but at least WR 225 448 is known to have such an effect (Peters & Robinson 1984b). WR 225 448 showed little cross-resistance with primaquine against the asexual blood stages of the primaquine-resistant P line of *P. berghei* (Peters et al 1984a). Studies of WR 225 448 provided evidence of a significantly

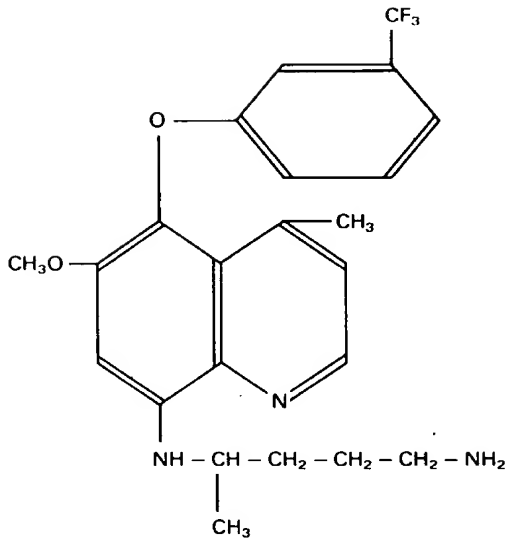


Fig. 51.22 WR 225 448

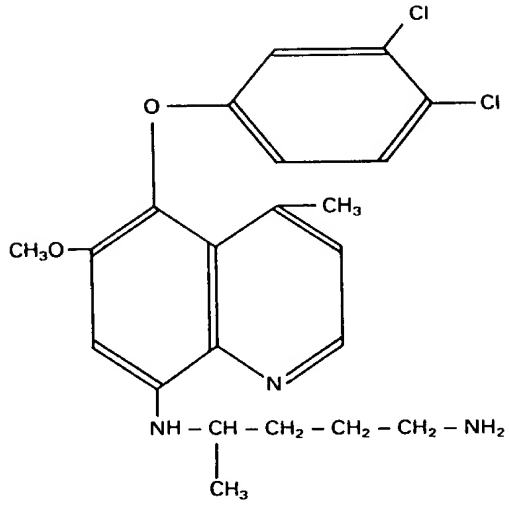


Fig. 51.24 WR 233 078

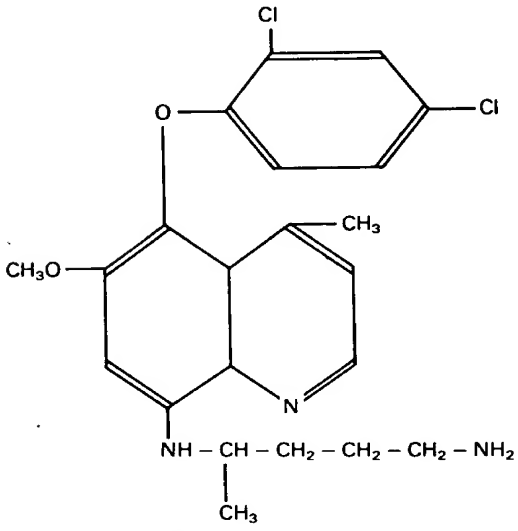


Fig. 51.23 WR 233 195

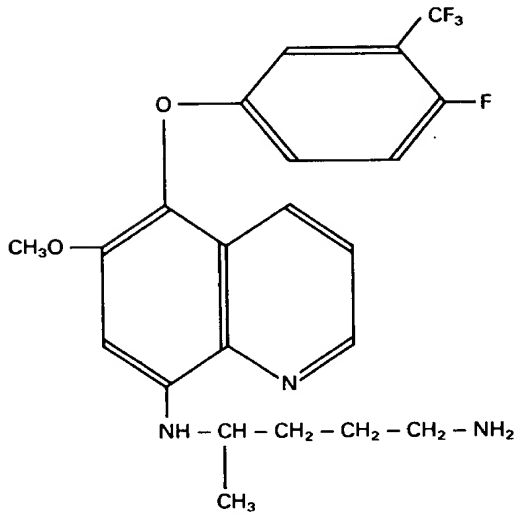


Fig. 51.25 WR 242 471

higher blood schizontocidal activity than primaquine also in the *P. cynomolgi/Macaca mulatta* and the *P. vivax/Aotus trivirgatus* models.

Although the acute toxicity of this compound in rodents is less than that of primaquine, the chronic toxicity appears to be higher and the haematotoxicity is more marked than with primaquine (Sweeney et al 1983). It remains to be seen whether the more recent compounds WR 233 078, WR 233 195 and especially WR 242 471 are better tolerated. Any improvement of the chemotherapeutic index over that of primaquine would, indeed, make the use of the 8-aminoquinolines more practicable.

Analogous to the 5-phenoxy derivatives of primaquine, a series of 5-phenylthio and 5-anilino substi-

tuted compounds were synthesized and tested (Tanabe et al 1978). All of the 5-phenylthio derivatives were radically curative, but at higher dose levels than with primaquine. However, they were non-toxic in mice at 640 mg/kg. There was no blood schizontocidal activity in *P. berghei*-infected mice at this dose. The 5-anilino derivatives were generally less active than the 5-phenylthioprimaquines. Tanabe et al (1978) concluded that the aromatic substitution in the 5 position reduces the toxicity of primaquine, but the best hope for high activity lies with the 5-phenoxy substituted derivatives.

Following the observation that the tissue schizontocidal activity of 4-methylprimaquine is approximately twice that of primaquine, LaMontagne et al (1982) synthesized a series of 5-alkoxy analogues of 4-methylprimaquine. The 5,6-bis(alkoxy)-8-aminoquinolines generally showed a higher suppressive activity against *P. berghei* in mice as compared to primaquine and 4-methyl-primaquine, but several members of this group also demonstrated radical curative activity against *P. cynomolgi* in rhesus monkeys. The most active compounds, in this respect, were two (5,6-dimethoxy-4-methyl-8-quinolinyl)-hexanediamines. The toxicity of these new compounds appears to be similar to that of primaquine, but the higher radical curative effect and suppressive action at relatively low doses would justify further investigation of this group.

The *P. cynomolgi/Macaca mulatta* screen for radical curative activity has played a key role in the determination of structure/activity relationships of 8-aminoquinolines, an area that has been pioneered by Schmidt (1983a, b). His studies led to the identification of 8-aminoquinolines with high primaquine indices, which may ultimately result in a simplification of antirelapse treatment of vivax malaria, ideally in the form of single-dose administration. To this purpose it would be worth exploring potential synergists such as mirincamycin, a lincomycin derivative, which was found to produce a significant enhancement of the radical curative activity of primaquine in the *P. cynomolgi/M. mulatta* system (Schmidt 1985).

4-quinolinemethanols

The first 4-quinolinemethanols were synthesized during the Second World War, but they were not immediately followed up. Thus more than two decades elapsed between the synthesis of α[(dibutylamino)methyl]-8-dichloro-2-(3′,4′-dichloro) phenyl-4-quinolinemethanol (Lutz et al 1946) and its further investigation in the research programme of the Walter Reed Army Institute of Research under code number WR 30 090 (Rothe & Jacobus 1968). The compound (Fig. 51.26) proved to be highly effective against induced falciparum malaria when given at a dose of 230 mg every eight hours for six days (Martin et al 1973), but the response was to some extent compromised by cross-resistance to chloroquine. Studies in vivax malaria proved that WR 30 090 had activity against the asexual blood stages, but not against the exoerythrocytic stages of the parasite. The drug was well tolerated and caused little photosensitivity.

Hall et al (1975a) compared the activity of WR 30 090 with that of quinine and of a 9-phenanthrenemethanol (WR 33 063) in 207 patients in south-eastern Thailand, where chloroquine-resistant *P. falciparum* is a major problem. Quinine cleared parasitaemia within the shortest time, but the effect of WR 30 090 was almost as fast, followed by WR 33 063 (mean clearance times 70, 72 and 77 hours respectively). However, WR 33 063 produced the highest cure rate in falciparum malaria, followed by WR 30 090 and quinine (92%, 86% and 85% respectively). WR 33 063 was the best tolerated compound, quinine the least tolerated drug. Side effects of WR 30 090 included headache, backache and urticaria. WR 30 090 has also suppressive activity against chloro-

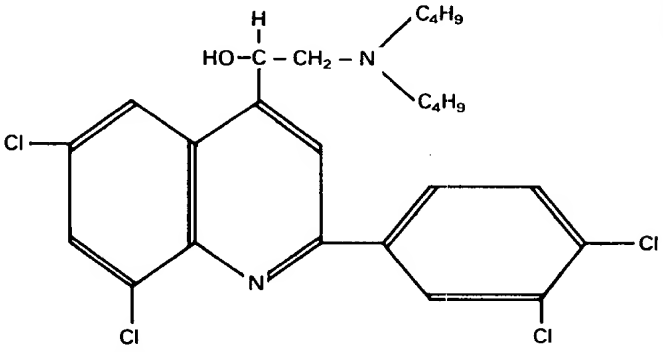


Fig. 51.26 WR 30 090

quine- and pyrimethamine-resistant *P. falciparum*, and against *P. vivax* when given weekly at doses of 800 mg, 690 mg or 460 mg. Suppressive cure was observed in 20 out of 26 men infected with *P. falciparum* (Clyde et al 1973).

WR 30 090 was superseded by a series of 4-quinolinemethanols which structurally resemble quinine. The most prominent representative of this new group, WR 142 490 or mefloquine (Fig. 51.27), was recently registered and is now being used in areas with multiresistant falciparum malaria. Details on this drug are given in Chapter 31. The development of mefloquine, initiated by the Walter Reed Army Institute of Research, is an example of an effective and mutually useful interaction between this Institute, WHO and industry in the areas of preclinical studies, drug formulation and galenics, pharmacokinetics and clinical trials.

Based on past experience with chloroquine and the sulfadoxine/pyrimethamine combination, the Steering Committee of the Scientific Working Group on the Chemotherapy of Malaria (UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases) recommended that steps be taken to prevent the development of resistance to mefloquine for as long as possible. This may be achieved by restricting the use to radical treatment and to areas where chloroquine resistance justifies the deployment of this medicament. On the other hand it was shown in rodent malaria models that the combination with sulfadoxine and pyrimethamine could delay the advent of drug resistance to the individual components. The drugs have at least an additive effect. Since at present the sulfadoxine and pyrimethamine half-lives are those which come closest to that of mefloquine—though not identical—this combination was selected for further development (UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases 1983). After the essential preclinical studies, a series of phase I trials for tolerability and pharmacokinetics was carried out with combination tablets containing 250 mg mefloquine (base), 500 mg sulfadoxine and 25 mg pyrimethamine each. The trials demonstrated that the plasma half-lives of the individual compounds were not substantially altered by the presence of the others. The combination was well tolerated. Clinical phase II and III studies have largely been completed (WHO 1984b). A large-scale curative field trial with the mefloquine/sulfadoxine/pyrimethamine combination, involving approximately 100 000 patients with falciparum malaria in an area with multiresistant parasites was conducted in Thailand in 1983–85 in cooperation between the Government of Thailand, the pharmaceutical industry and WHO. This is the largest field trial ever undertaken with an antimalarial drug.

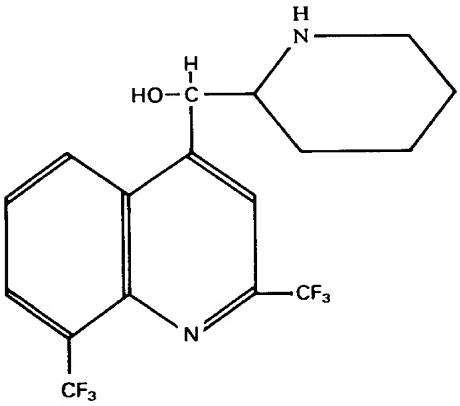


Fig. 51.27 WR 142 490 (Mefloquine)

It is envisaged that the mefloquine/sulfadoxine/pyrimethamine combination will be used exclusively for the radical treatment of falciparum malaria in areas with chloroquine-resistant *P. falciparum*, with the exception of pregnant women, infants up to six months and persons not tolerating sulfonamides. These individuals should be treated with mefloquine alone. It is not foreseen to use this combination prophylactically in view of the contraindications governing the use of long-acting sulfonamides (risk of adverse reactions). The large prophylactic deployment of mefloquine in endemic areas is not advocated on account of the risk of accelerating the development of drug resistance. Nevertheless, it will be unavoidable that mefloquine (monocompound) will be used for short-term chemosuppression, especially by non-immune travellers to areas with intensive malaria transmission. In this connection it would be wise to remember that the side effects of mefloquine are of the 'quinine-type'. It remains to be seen to what extent they interfere with physiologi-

cal performance, e.g. in aircraft personnel (Stockwell 1982); only when appropriate investigations have been completed will it be possible to provide guidelines for the use of mefloquine for malaria prophylaxis in occupational groups whose professional performance may be detrimentally influenced by the drug.

The induction of mefloquine resistance in *P. berghei* is relatively easy, but the parasite apparently reverts quickly to a sensitive status after drug pressure is relieved (Puri et al 1982). In *P. falciparum* in vitro it is more difficult to produce a loss of sensitivity to a level indicative of resistance, although Brockelman et al (1981) have succeeded in doing so. This may have been an isolate-specific event since several other laboratories experienced major difficulties in the same endeavour. Unfortunately, the loss of the isolate precludes further investigation. There is no doubt that primary resistance to mefloquine occurs in nature, and the cases described by Boudreau et al (1982) and Bygbjerg et al (1983) indicate the presence of such parasite populations in South-East Asia and in East Africa. Similar findings have started to emanate from in vitro studies, but the frequency of primary resistance appears to be quite low. It indicates, however, that appropriate measures must be taken to prevent the propagation of such infections.

The identification of mefloquine as a candidate compound for further development did not preclude the investigation of other 4-quinolinemethanols; there are two compounds of interest, namely α-(tert-butylaminoethyl)-2,8-bis-(trifluoromethyl)-4-quinolinemethanol (WR 184 806) (Blumbergs et al 1975) and α-(2-piperidyl)-2-trifluoromethyl-6,8-dichloro-4-quinolinemethanol (WR 226 253) (Pinder & Burger 1968). WR 184 806 has the basic ring structure and the trifluoromethyl substitutions of mefloquine, but an alkylaminoalkyl substitution instead of the piperidine moiety in the 4-position (Fig. 51.28). WR 226 253 resembles mefloquine even more in as much as chlorine is substituted in the 8-position in the place of the trifluoromethyl moiety; an additional chlorine substitution is found in the 6-position (Fig. 51.29). Schmidt et al (1978b) studied these compounds in *Aotus trivirgatus* with induced *P. falciparum* and *P. vivax* infections and found them to be highly effective against these plasmodia. In multidrug-resistant *P. falciparum*, WR 184 806 was one-third as effective and WR 226 253 twice as effective as mefloquine. Both are more active against *P. vivax* than against *P. falciparum*. The curative, blood schizontocidal effect is a function of the total dose; a single dose was as effective as the same total dose given over three or seven days. Moreover, and in contrast with mefloquine, WR 184 806 (as the phosphate salt) could be given through the intravenous route, and the full curative dose could be administered in one i.v. injection.

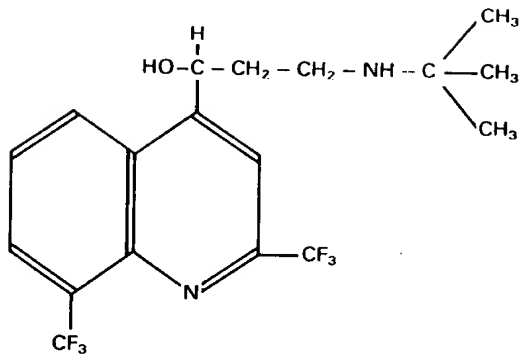


Fig. 51.28 WR 184 806

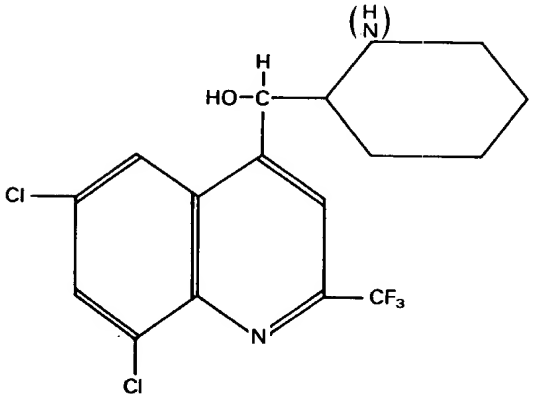


Fig. 51.29 WR 226 253

Quinolones

The first indication of an antimalarial activity of 4-quinolones came from endochin [2-methyl-3n-

heptyl-7-methoxy-4(1H)-quinolone]. It possesses causal prophylactic and blood schizontocidal activity in canaries infected with *P. praecox*, but clinical trials produced disappointing results. Some 350 quinolones were synthesized by Imperial Chemical Industries Ltd in the course of a research programme aiming at the development of effective coccidiostatic agents. Several of these compounds also showed antimalarial activity which did not necessarily parallel the coccidiostatic effect.

The basic structure of these compounds has a remarkable similarity with clopidol (Fig. 51.30). Three of the quinolones, ICI 56 780, ICI 59 350 and ICI 60 128, showed a very high activity against *P. berghei* in mice. Their acetoxy derivatives (ICI 61 166, ICI 59 351, ICI 61 523) were generally even more active. Ryley & Peters (1970) performed detailed studies with ICI 56 780 and ICI 60 128 (Figs. 51.31 & 51.32).

In the suppressive test, using the *P. berghei* N strain in mice, ICI 56 780 showed high blood schizontocidal activity with an ED_{50} of 0.05 mg/kg and an ED_{90} of 0.15 mg/kg. The ED_{50} and ED_{90} values for chloroquine phosphate are 2.5 and 3.9 mg/kg respectively. The LD_{50} of chloroquine phosphate in mice is 120 mg/kg while that of ICI 56 780 was > 5 g/kg.

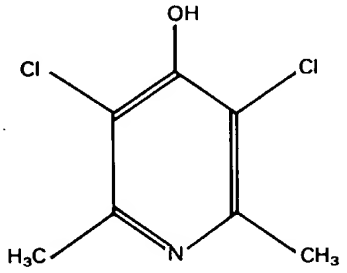


Fig. 51.30 Clopidol (Meticlorpindol)

In curative experiments with established *P. berghei* (N strain) infections in mice, single-dose treatment (s.c.) with ICI 56 780 at doses of 50 and 500 mg/kg produced a reduction of parasitaemia to submicroscopic levels by day 4, but all infections recrudesced by day 10 and all mice died between days 14 and 20. Causal prophylactic activity in both *P. berghei* and *P. yoelii* was observed with single s.c. doses of 30 mg/kg administered one hour after sporozoite inoculation.

A high degree of resistance against ICI 56 780 was selected in the course of one passsage, following unsuccessful blood schizontocidal treatment of a *P. berghei* infection. The same was found with ICI 60 128. The resistance proved to be very stable in the course of numerous mouse passages over a period of two years.

The type of activity of ICI 56 780 was apparently quite different from that of 4-aminoquinolines, DHFR and DHPS inhibitors; there was also no antagonistic effect seen with common antagonists to DHFR and DHPS inhibitor activity. However, there was marked synergism between chlorcycloguanil and ICI 56 780, between sulfadoxine and ICI 56 780, and between sulfadiazine and ICI 60 128.

While no antimalarial effect of ICI 60 128 was seen in the *P. gallinaceum*/chicken model (ICI 56 780 was not tested), evidence of a strong causal prophylactic effect was found in the very limited experiments in rhesus monkeys infected with *P. cynomolgi*. Although the quinolones do not,

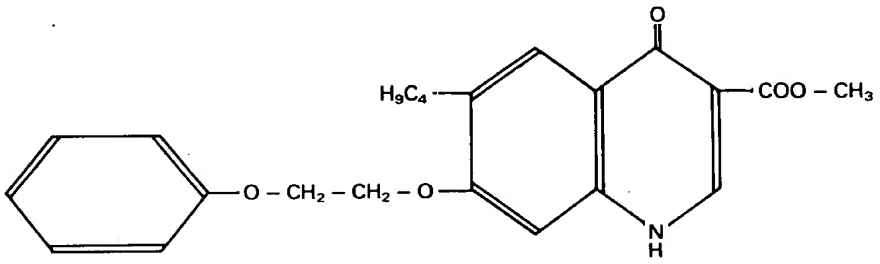


Fig. 51.31 ICI 56 780

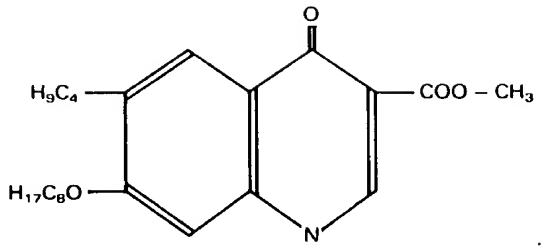


Fig. 51.32 ICI 60 128

on first sight, appear attractive as candidates for further development, it should be borne in mind that they are generally well-tolerated compounds. Their causal prophylactic activity and the possibility of potentiating their blood schizontocidal effect to radical curative activity in various animal models through their combination with a suitable partner merit further investigation. The quinolones may also serve as an example of the relative merits and deficiencies of various screening approaches. If the customary Rane test had been the only one employed, Ryley & Peters (1970) would have seen very little activity beyond a significant prolongation of the survival time of the rodents. This may also explain the relatively modest antimalarial activity of another series of 4(1H)-quinolones reported by Casey (1974).

Naphthoquinones

The antimalarial activity of naphthoquinones was recognized when a series of 2-hydroxy-1,4-naphthoquinones was investigated in the mid 1940s. Two compounds, M 1916 and M 285, were noted for their suppressive and curative activity against *P. lophurae* and *P. gallinaceum*, but showed disappointingly low activity against human vivax and falciparum malaria (Fieser et al 1948). Fawaz & Haddad (1951) had success, nevertheless, with intravenously administered lapinone (M 2350) for the treatment of vivax malaria. Following up on the earlier observations in avian malaria systems, Fieser et al (1967a, b) investigated various 2-hydroxy-3-(ω-cyclohexylalkyl)-1,4-naphthoquinones and 2-hydroxy-3-[ω(1-adamantyl)alkyl]-1,4-naphthoquinones for their activity against *P. berghei* in mice, using oral administration. Some antimalarial activity was found with members of these groups.

Berberian & Slighter (1968) conducted studies in which the structure/activity relationships of 32 compounds belonging to five distinct series of alkyl and substituted alkyl 2-hydroxy-1,4-naphthoquinones were investigated. The compounds which were given intragastrically (i.g.) were evaluated for their suppressive and curative effects in blood-induced infections of *P. berghei* in mice. The suppressive effect in the 3-cyclohexylalkyl-2-hydroxy-1,4-naphthoquinones was highest (ED_{50} 12.4 mg/kg as compared to chloroquine 4.65 mg/kg) when the alkyl chain contained 8 carbon atoms. In the 3-adamantylalkyl-2-hydroxy-1,4-naphthoquinones, the highest activity was obtained with a short length of the alkyl chain, 2 carbon atoms being optimal (ED_{50} 15.6 mg/kg). In the 3-n-alkyl series a chain length of 9 carbon atoms (ED_{50} 19 mg/kg) and in the 3-phenylalkyl group a chain length of 8 carbon atoms proved to be optimal (ED_{50} 39 mg/kg). The 3-isoalkyl derivatives yielded results quite similar to those of the 3-n-alkyl series. While the suppressive effect was marked, there were only three compounds which exhibited measurable curative activity, namely 3-(8-cyclohexyloctyl)-2-hydroxy-1,4-naphthoquinone, 3-(9-cyclohexylnonyl)-2-hydroxy-1,4-naphthoquinone and 3-(8-isodecayl)-2-hydroxy-1, 4-naphthoquinone. However, Berberian & Slighter (1968) observed a marked effect on tissue forms of *P. berghei*. Naphthoquinones were also investigated by the Walter Reed Army Institute of Research which studied 3-(8-cyclohexyloctyl)-2-hydroxy-1, 4-naphthoquinone (Fig. 51.33) in patients infected with the Malaya (Camp) isolate of *P. falciparum* (WHO 1973). This compound (menoctone, WR

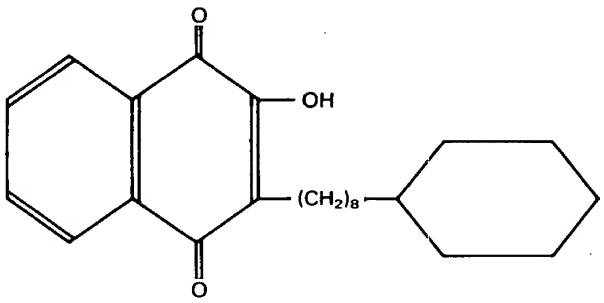


Fig. 51.33 WR 49 808 (Menoctone)

49 808) exerted only slight blood schizontocidal activity upon oral administration of daily doses of 0.4–0.5 g for three days. With this regimen the drug did not show any appreciable gametocytocidal or sporontocidal effect. It also had no causal prophylactic activity against the Malaya (Camp) or Uganda I isolates of *P. falciparum*. It was concluded that 'poor absorption from the gastrointestinal tract is characteristic of compounds of this group and may explain the lack of demonstrable activity by WR 49 808' (WHO 1973). This brought further investigation of the naphthoquinones to a temporary halt. At the time, no attention seems to have been paid to the observations of Fawaz & Haddad (1951) which should have triggered studies on their bioavailability that could have explained the disappointingly low activity of the earlier formulations in human malaria.

Recently, interest in hydroxynaphthoquinones was renewed. One compound, 2-(4-t-butylcyclohexyl)-3-hydroxy-1,4-naphthoquinone (BW 58C) was shown to be highly active against *P. falciparum* in vitro and against *P. berghei* and *P. cynomolgi* in vivo (Hudson et al 1985). It was also effective against *Theileria parva, Theileria annulata* and *Eimeria tenella*. In the *P. yoelii nigeriensis*/mouse system BW 58C exhibited causal prophylactic activity comparable to that of primaquine. Chloroquine-, mefloquine-, pyrimethamine-, sulfonamide-, and primaquine-resistant lines of *P. berghei* were shown to be fully susceptible to BW 58C. The compound has a low mammalian toxicity, with an oral LD_{50} in rats of > 2 g/kg body weight.

Quinazolines

The first member of this group of DHFR inhibitors to be tested for antimalarial activity was 2,4-diamino - 6 - (3,4-dichlorobenzylamino)quinazoline (Fig. 51.34) which had been synthesized by Davoll (1966). It has suppressive blood schizontocidal activity against *P. berghei* in the mouse at a dose of 200 mg/kg i.g. and against *P. cynomolgi* and *P. knowlesi* in *Macaca mulatta* at a dose of 100 mg/kg i.g. It acts synergistically with sulfadiazine (Thompson et al 1969). There was no radical cure when the compound was given at the above dose levels for six days in mice or for five days in rhesus monkeys. The compound was more effective against pyrimethamine-resistant *P. berghei* than against the sensitive parent strain, but there was an indication of reduced sensitivity in cycloguanil-resistant *P. berghei*.

The next compound to be extensively investigated was 2,4-diamino-6-[(3,4-dichlorobenzyl)nitrosamino]quinazoline in the form of the base or the acetate (Thompson et al 1970). Mean daily oral doses of 0.74 mg/kg of the base and 1 mg/kg of the acetate were 100% suppressive in blood infections of *P. berghei* in mice treated for five days. The SD_{90} for both formulations was approximately 0.4 mg/kg per day upon oral administration, but 0.2 mg/kg for the base and 0.08 mg/kg for the acetate when the drugs were given i.g. This difference may be ascribed to Tween 80 which had been used for preparing the aqueous gavage fluid. There was no radical cure of established *P. berghei* infections after single oral doses of 12.5–100.0 mg (acetate)/kg. However, radical cure was achieved with two oral doses of 50 mg/kg in one day, two oral doses of 25 mg/kg per day for two days, and two oral doses of 12.5 mg per day for four days. There was no sign of cross-resistance in chloroquine- and pyrimethamine-resistant lines of *P. berghei*, but sensitivity was reduced in

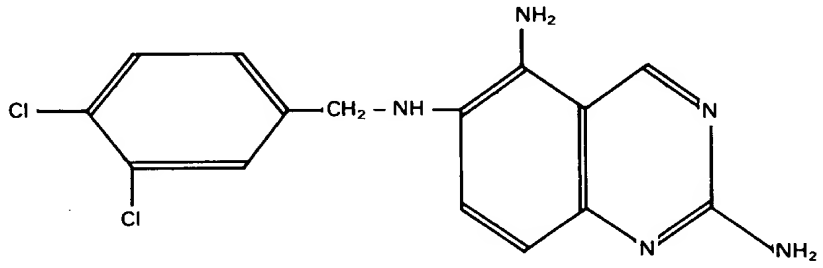


Fig. 51.34 2,4-diamino-6-(3,4-dichlorobenzylamino) quinazoline

cycloguanil- and sulfone-resistant lines in the suppressive tests. A moderate degree of antagonism between the acetate compound and PABA was evident, but no antagonism was found with folinic acid or sodium folate. The base compound was not tested for PABA or for folate antagonism. Synergism of DDS with 2,4-diamino-6-[(3,4-dichlorobenzyl)nitrosamino] quinazoline acetate is marked and is also observed with DDS-resistant isolates of *P. berghei*. Both formulations of the compound given at a single i.m. dose of 400 mg/kg in 40% benzyl benzoate and 60% castor oil had a repository antimalarial effect in mice, with strong suppression during the first three weeks and significant activity lasting for at least nine weeks. In the early experiments in the *P. cynomolgi/Macaca mulatta* system, the base compound was apparently curative at an oral dose of 2.5 mg/kg daily for five days, and at a single intramuscular dose of 10 mg/kg. The acetate was used at a total oral dose of 50 mg/kg in five different dose regimens ranging from a single dose up to 16 doses over eight days. Radical cure was observed in 14 out of the 15 treated monkeys (Thompson et al 1970). Both formulations of the compound exerted prolonged repository action in monkeys when given i.m. at a dose of 50 mg/kg in benzylbenzoate-castor oil or isopropyl mystirate-peanut oil. There was uniform protection against *P. cynomolgi* challenge for 105 days and some animals were protected for more than seven months.

The results of subsequent studies on the acetate salt of 2,4-diamino-6-[(3,4-dichlorobenzyl)-nitrosamino]quinazoline (Schmidt & Rossan 1979) in the *P. cynomolgi/Macaca mulatta* system were less promising inasmuch as daily doses of 2.0–50.0 mg/kg for seven days failed to radically cure monkeys infected with the drug-sensitive RO isolate and the pyrimethamine-resistant RO/PM isolate of *P. cynomolgi*. However, clearance of the blood infection was invariably obtained with all dose levels in infections with the RO isolate, while daily doses of >2 mg/kg were required to clear the blood infections with the RO/PM isolate. Contrary to the observations in *P. berghei*-infected mice, pyrimethamine-resistant *P. cynomolgi* was less sensitive to the quinazoline than the parent line. Further observations showed that infections which had recrudesced after treatment with subcurative doses of the quinazoline did not respond to normally curative doses of the compound. This was found to be due to the emergence of quinazoline-resistant parasites. This resistance proved to be stable through mosquito transfer.

The Malaria Research Group of the Third Army Medical College, Ghongquing, China (1982) investigated the effect of 2,4-diamino-6-[(3,4-dichlorobenzyl)nitrosamino] quinazoline on the sporogonic stages of malaria parasites. *Aedes albopictus* fed on *P. gallinaceum*-infected chickens having received 2 mg/kg of the compound or 1 mg/kg of the 1:1 mixture of the compound and dapsone failed to develop infections of the salivary glands. Similar results were obtained in the *P. cynomolgi/Anopheles stephensi* or *A. balabacensis* systems, and in *A. balabacensis* membrane-fed with blood from patients with vivax and falciparum malaria who had been treated with the compound. *P. falciparum* gametocytes which had emerged after the disappearance of the asexual forms following quinazoline treatment were apparently non-infective for *A. balabacensis*. The sporontocidal effect of the quinazoline was ascribed to preventing the passage of the ookinetes through the peritrophic membrane or to an interference with DNA replication in the oocysts. This group also briefly reported on the clinical use of this quinazoline alone or in association with dapsone (1:1) for the treatment of human vivax and falciparum malaria where it was found to be effective. Results from the prophylactic use of this quinazoline seem to indicate that it may block malaria transmission through the inhibition of sporogony.

Chinese workers (Dai et al 1982) synthesized a similar compound 2,4-diamino-6-[(4-chlorobenzyl)-N-methylamino]quinazoline and various of its salts. These compounds showed relatively modest and rather short-lasting suppressive activity in the rodent malaria models used. The effects were even less marked in the *P. cynomolgi/Macaca mulatta* system. There was no radical curative activity in either experimental model.

Following the earlier leads, a great number of quinazoline derivatives were synthesized and screened in the 1970s. These included 2,4-diamino-6-[(aralkylmethyl)-amino]quinazolines

and 2,4-diamino-6-[(heterocyclicmethyl)-amino] quinazolines (Davoll et al 1972; Elslager et al 1972), 2,4-diamino-6-[aralkyl and alicyclic)thio-, sulfinyl-, and sulfonyl]quinazolines (Elslager et al 1978a), and 2,4-diamino-6-[(phenyl- and naphthyl) thio]quinazolines (Elslager et al 1978b). Active compounds were found in all groups, but the 2,4-diamino-6-[(aralkyl and alicyclic) thio-, sulfinyl-, and sulfonyl]quinazolines were the relatively least effective. Several of the 6-thio-,6-sulfinyl-, and 6-sulfonyl-substituted 2,4-diaminoquinazolines were investigated to determine their radical curative activities against the chloroquine-resistant Vietnam Oak Knoll isolate and the pyrimethamine-resistant Malayan Camp-CH/Q isolates of *P. falciparum* in *Aotus trivirgatus* (Schmidt 1979a). In these studies 2,4-diamino-6-[(5-trifluoromethylphenyl)-thio]quinazoline (WR 159 412)(Fig. 51.35) and 2,4-diamino-6-(2-naphthylsulfonyl)quinazoline (WR 158 122)(Fig. 51.36) were found to be the most active compounds, effecting radical cure with the Vietnam Oak Knoll isolate at remarkably low doses (WR 158 122 CD_{90} = 0.025 mg/kg and WR 159 412 CD_{90} = 0.1 mg/kg daily i.g. for seven days). The CD_{90} was significantly higher (0.63 mg/kg for WR 159 412 and >3 mg/kg for WR 158 122) upon retreatment in monkeys which had been unsuccessfully pretreated with the same compounds. The CD_{90} levels with the pyrimethamine-resistant Malayan Camp CH/Q isolate were a priori higher (WR 158 122: 1.2 mg/kg and WR 159 412: 0.45 mg/kg daily i.g. for seven days); unsuccessful pretreatment led to a significant rise of the CD_{90} also with this isolate.

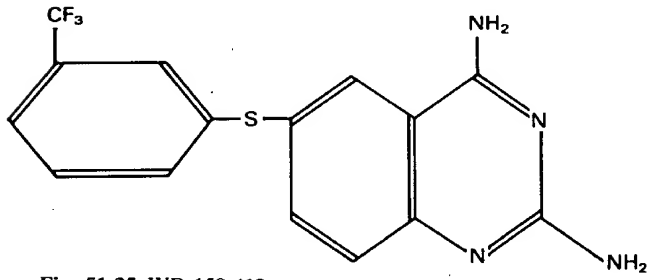


Fig. 51.35 WR 159 412

Several 2,4-diamino-6-N^1,N^2 disubstituted hydrazinoquinazolines showed suppressive activity against *P. berghei* in mice and causal prophylactic activity against *P. yoelii* in mice, several of them at oral doses of 2.5 mg/kg body weight for three consecutive days (Zheng et al 1983). Yao et al (1984) explored a series of α(alkylaminomethyl)-2-phenyl-4-quinazolinemethanols and found only modest activity against *P. berghei* in mice with two out of 14 compounds.

In view of the earlier observed strong potentiation of quinazoline activity by PABA antagonists in the *P. berghei*/mouse model, Schmidt (1979b) investigated combinations of sulfadiazine and WR 158 122 or WR 159 412 in *Aotus trivirgatus* infected with various drug-sensitive and drug-resistant isolates of *P. falciparum* and *P. vivax*. Marked synergism was found between sulfadiazine and the two quinazolines: the activity was enhanced 7 to 75-fold with WR 158 122 and 5 to 13-fold with WR 159 412, maximal increases of activity being observed at only 5 mg sulfadiazine/kg per day. Although there was still a significant difference between the CD_{90} levels against pyrimethamine-sensitive and pyrimethamine-resistant isolates, the combination of sulfadiazine and acceptably small doses of WR 158 122 was curative in infections with the most pyrimethamine-resistant isolates. The combination of sulfadiazine with WR 158 122 or WR 159 412 prevented also the emerg-

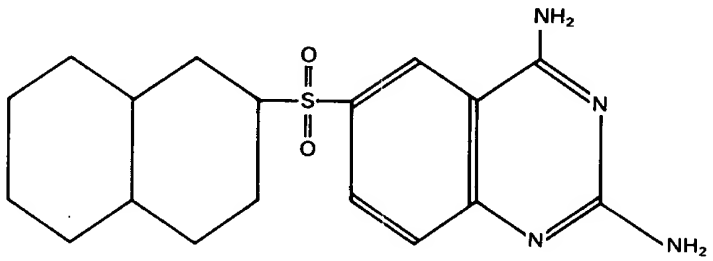


Fig. 51.36 WR 158 122

ence of quinazoline resistance. Observations in *P. berghei*-infected mice suggested synergism between WR 158 122 and its tetrahydro-analogue WR 180 872 (Kinnamon et al 1976). Further investigations in the *P. cynomolgi/Macaca mulatta* system gave some evidence of potentiation, which was, however, far less than in the rodent model (Kinnamon & Davidson 1980).

The limited clinical evaluation of WR 158 122 in volunteers infected with the fully sensitive Uganda I isolate of *P. falciparum* was far less encouraging than the results of the owl monkey studies. Daily doses of up to 250 mg failed to produce radical cure in humans, while daily oral doses of 1000 mg for three days cured only one in three infections (Schmidt 1979b; Bruce-Chwatt et al 1981). The drug was well tolerated at the highest dose tested, i.e. 1300 mg daily for three days. From blood level studies using a microbiological assay method, it appears that poor absorption from the gastrointestinal tract in man, quite in contrast to *Aotus*, is responsible for the disappointing clinical results. However, this problem could probably be solved through appropriate galenic formulation.

Dihydrotriazines

A series of substituted N-oxydihydrotriazines synthesized by Beecham Research Laboratories as potential antimicrobial agents (Mamalis et al 1965) were found to possess also a potent effect against *P. berghei* in mice (Knight & Peters 1980). One of the most active of these compounds was 4, 6-diamino-1,2-dihydro-2,2-dimethyl-1(3,4 dichlorobenzyloxy)-1,3,5-triazine hydrochloride (clociguanil, BRL 50 216, WR 38 839)(Fig. 51.37). It was explored experimentally both within and outside the research programme of the Walter Reed Army Institute of Research, and reached the stage of preliminary clinical investigation, but, since the compound did not seem to offer major advantages over pyrimethamine with which it shared cross-resistance, investigations were terminated. In primary screening for blood schizontocidal activity in the *P. berghei*/mouse model, WR 38 839 showed limited curative activity at the 20 mg/kg single dose level, and 100% cure at single doses of 80 mg/kg using a drug-sensitive isolate of *P. berghei* (Knight & Peters 1980). The ED_{50} of 0.16 mg/kg was less than one-tenth of that of cycloguanil or chloroquine. The difference with cycloguanil was even more marked at the ED_{90} level (0.39 mg/kg as opposed to 13.5 mg/kg), but remained approximately the same with regard to chloroquine. The acute toxicity in mice was less than one-tenth of that of cycloguanil hydrochloride and chloroquine diphosphate (LD_{50} >2500 mg/kg as opposed to 220 mg/kg and 200 mg/kg respectively).

In a therapeutic test in mice infected with the N isolate of *P. berghei* and showing a starting parasitaemia of 8–9%, the s.c. administration of 100 mg/kg of WR 38 839 eliminated parasitaemia within 72 hours, but recrudescences occurred after 196 hours. Cycloguanil and chloroquine, at this dose level, did not eliminate parasitaemia. Similar results were found after the oral administration of the same doses, but following treatment with WR 38 839 parasitaemia reappeared within 168 hours. The compound proved to exert full causal prophylactic action in the *P. berghei*/mouse system at single doses of 10, 3 and 1 mg/kg given s.c. 45 minutes after sporozoite inoculation. In-

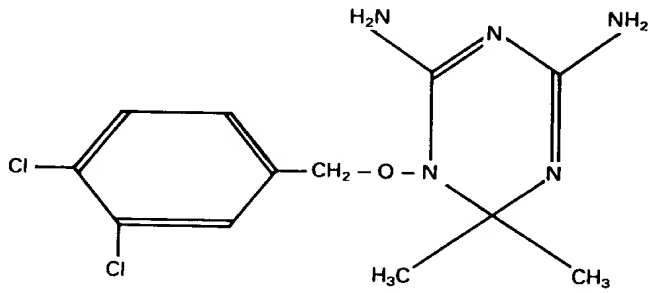


Fig. 51.37 Clociguanil (WR 38 839; BRL 50 216)

terestingly, there was little sign of cross-resistance between pyrimethamine and WR 38 839 in the rodent malaria models, but a marked degree of cross-resistance with fully cycloguanil-resistant isolates. There was marked potentiation of the blood schizontocidal effect with sulfadimethoxine which was also evident against cycloguanil-resistant isolates.

In studies on induced resistance in *P. berghei*, Knight & Williamson (1980) found it more difficult and time-consuming, yet possible, to induce resistance to WR 38 839 as compared to cycloguanil with which it shared cross-resistance. This was ascribed to the significantly more steep dose response to WR 38 839 (Knight & Peters 1980). Still, the WR 38 839-resistant isolate was sensitive to pyrimethamine.

WR 38 839 has been employed in clinical trials in non-immune volunteers. In infections with the drug-sensitive Uganda I isolate of *P. falciparum* radical cure was achieved by oral treatment with 0.6 g daily for three days. Approximately double this dose failed to cure infections with the resistant Malayan Camp and Vietnam Marks isolates (Rieckmann et al 1971). When combined with sulfadiazine, WR 38 839 was curative in half the patients infected with these isolates. Moreover, the combination of these drugs proved to have a causal prophylactic effect against sporozoite-induced *P. falciparum* infections (Vietnam Marks isolate), while neither compound given alone at the same or higher doses prevented the occurrence of parasitaemia (Rieckmann et al 1971). In partially immune children in The Gambia where *P. falciparum* was pyrimethamine-sensitive at the time, WR 38 839 showed a marked blood schizontocidal activity (Laing 1971), which was potentiated by sulfadimethoxine and sulfafurazole (Laing 1974). But it was concluded that WR 38 839 did not offer a major advantage over pyrimethamine or proguanil. In the absence of pharmacokinetic studies and given the evidence that a concentration of 50 μg/l (approximately 0.15×10^{-6}M) significantly affects both the Vietnam Marks and the Malayan Camp isolates of *P. falciparum* in vitro (WHO 1973; Bruce-Chwatt et al 1981), problems seem to exist regarding the bioavailability of WR 38 839 after oral administration in man. Indeed daily doses of 0.6 g should have been conducive to establishing much higher plasma concentrations, unless there is a first pass phenomenon or an unusual tissue distribution of the compound.

Another dihydrotriazine, 4,6-diamino-1,2-dihydro-2,2-dimethyl-1-[(2,4,5 trichlorophenoxy) propyloxy]-1,3,5-triazine hydrobromide (WR 99 210, BRL 51 084) (Fig. 51.38) and the hydrochloride analogue (BRL 6231) were also synthesized by Beecham Research Laboratories (Knight et al 1982). The particular interest of these compounds is that they showed no cross-resistance whatsoever with pyrimethamine. In infections with the N strain of *P. berghei* both compounds were completely suppressive at dose levels of 10 and 1 mg/kg (4 times s.c.). The ED_{50} for blood schizontocidal activity in *P. berghei*-infected mice was 0.16 mg/kg (s.c. × 4) for BRL 6231, as compared to 0.19 mg/kg for pyrimethamine, 2.0 mg/kg for cycloguanil hydrochloride and 2.3 mg/kg for chloroquine diphosphate. At the ED_{90} level the compound was equivalent to pyrimethamine (0.34 mg/kg as opposed to 0.35 mg/kg), but the oral ED_{90} for BRL 6231 was 20 mg/kg under the same test conditions, indicating

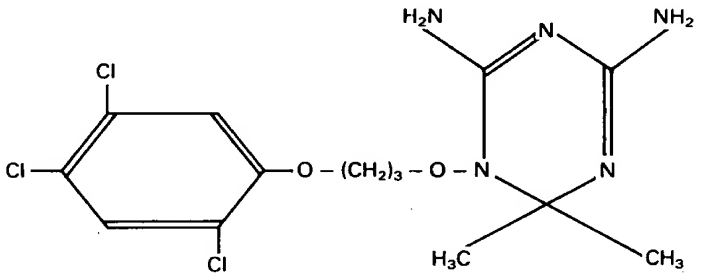


Fig. 51.38 WR 99 210 (BRL 51 084)

poor bioavailability of the drug when given orally. The acute toxicity of BRL 6231 was less than that of cycloguanil or chloroquine when given in a single dose, but higher when the same total dose was given in four repeated daily doses. The CD_{50} in the owl monkey/*P. falciparum* model was 6.1 mg/kg for WR 99 210 as compared to 35 mg/kg for WR 38 839 (Bruce-Chwatt et al 1981). WR 99 210 proved to be completely protective in the causal prophylactic test in the *P. berghei*/mouse model when given at 10, 3 and 1 mg/kg (single dose s.c.). In rhesus monkeys infected with sporozoites of *P. cynomolgi* WR 99 210 was given for 10 days at daily s.c. doses of 0.25, 0.50 and 1.00 mg/kg as from one day before sporozoite challenge. The two lower dose levels, although prolonging patency beyond day 37, were not fully protective, but the highest dose level was fully effective. There was no parasitaemia upon splenectomy. Primaquine at 1 mg/kg in the same regimen proved to be equally effective.

Studies of the resistance patterns in *P. berghei* (Knight et al 1982) showed that there was no cross-resistance between BRL 6231 and pyrimethamine, and only a very modest reduction of sensitivity to BRL 6231 in a cycloguanil-resistant line. In vitro studies conducted by Rieckmann (WHO 1973) showed incipient activity of WR 99 210 against the Malayan Camp and Uganda I isolates of *P. falciparum* at 0.5 μg/l (approximately 1.3×10^{-9}M) and a very high degree of growth inhibition in these two and the Vietnam Marks isolate at 2.5 μg/l (approximately 6×10^{-9}M). Resistance to BRL 6231 can be induced in *P. berghei* (Knight & Williamson 1982), but the process is much slower as compared to that with cycloguanil and pyrimethamine. BRL 6231-resistant lines of *P. berghei* show a low grade cross-resistance to cycloguanil and pyrimethamine.

Although WR 99 210 was poorly tolerated when given to healthy volunteers (Canfield & Rozman 1974), this group of compounds, especially WR 99 210 and BRL 6231, may well represent the most active DHFR inhibitors known to date. They would therefore deserve to be further investigated particularly as their mechanism of action is apparently only partially interrelated with that of other DHFR inhibitors. It is to be expected that their activity can be potentiated by DHPS inhibitors (e.g. sulfonamides) as known from earlier studies with dihydrotriazines. They thus hold promise especially for the practical exploitation of their causal prophylactic action. However, the most important obstacle to be overcome appears to be the poor bioavailability after oral administration. This problem may possibly be solved by the development of a suitable galenic formulation provided there is no first pass effect which would demand parenteral administration of the drug.

OTHER COMPOUNDS OF INTEREST

A great number of compounds are known to have an antimalarial effect, but many of these have not yet been exhaustively explored. Some of the substances listed in Table 51.6 may be worth further study. The majority, however, should be considered as lead compounds of chemical groups meriting further investigation. This is true, for example, of floxacrine (Fig. 51.39) the clinical development of which is precluded on account of the occurrence of endarteritis in dogs treated daily for two months or more at a malaria curative dose level. This may have limited significance if the drug is to be used for radical treatment only; but in a chronic toxicity screen, endarteritis is usually regarded as incompatible with registration for commercial introduction. Floxacrine could have been a potential candidate as a causal prophylactic drug, requiring extended administration at comparatively low doses, but this would nevertheless raise the issue of chronic toxicity. On the other hand, floxacrine may be regarded as a lead compound of a group which has not yet been extensively investigated. The synthesis of analogues and the careful evaluation of structure/activity and structure/toxicity relationships holds considerable promise for dissociating antimalarial and toxic effects.

Recently, the floxacrine group was further explored, and a new compound, 7-chloro-1-(4N-methylpiperazino-1N-imino) -10-hydroxy-1,2,3,4-tetrahydro-3-(4-trifluoromethylphenyl)-9(10H)-acridone was selected for further investigation (Raether & Mehlhorn 1984). This compound, S 825 455, was shown to be approximately 13

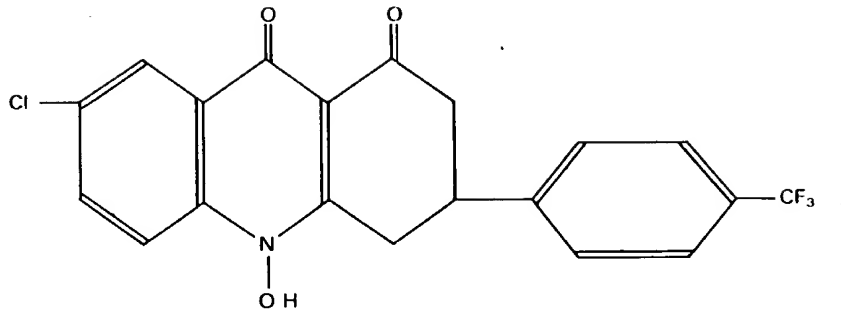


Fig 51.39 Floxacrine (WR 233 602)

times more effective than floxacrine in a blood schizontocidal *P. berghei*/mouse screen. Although the maximum tolerated oral dose of S 825 455 is lower than that of floxacrine the ratio of maximum tolerated to minimum curative dose was 256 for S 825 455 as compared to 80 for floxacrine. The difference was not as marked with s.c. administration. The minimum effective dose for oral administration (5 × 1.56 mg/kg) was lower than that for s.c. administration (5 × 3.12 mg/kg).

Another example of a detailed, systematic study of structure/activity relationship is the synthesis and evaluation of 121 guanidine compounds (Elslager et al 1974). Some 90 compounds were curative in the *P. berghei*/mouse model at single s.c. doses of 20–640 mg/kg, 62 were orally more active than quinine and 46 were 2–60 times as potent as the reference drug. Although the structure of the molecule would suggest that it would be a DHFR inhibitor, 1-(3,4-dichlorophenyl)-3-[4-[(1-ethyl-3-piperidyl)amino] -6-methyl-2-pyrimidinyl] guanidine (Fig. 51.40), the most promising of the new compounds, was fully active against *P. berghei* lines which were resistant to cycloguanil and DDS; however, there was some degree of cross-resistance to chloroquine. The compound was relatively well tolerated in various laboratory animal species and has yielded promising results in the *P. cynomolgi*/*Macaca mulatta* and *P. falciparum*/*Aotus* models; in the latter a curative effect was evident with the chloroquine-sensitive and pyrimethamine-resistant Palo Alto isolate of *P. falciparum*, whereas the Vietnam Monterey isolate proved to be resistant to the new candidate drug. These observations were probably instrumental in preventing the further development of this chemical group.

In a similar approach, Elslager et al (1969a, b, c) and Worth et al (1969) synthesized a large number of sulfanilylanilides and sulfonylanilides with the aim of developing repository drugs against malaria and leprosy. However, none of

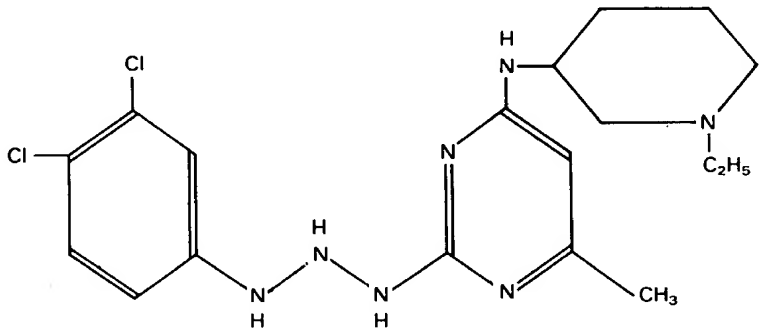


Fig. 51.40 1-(3,4-dichlorophenyl)-3-[4-[(1-ethyl-3-piperidyl) amino]-6-methyl-2-pyrimidinyl] guanidine

Table 51.6 Other compounds exhibiting antimalarial activity

Group	Name & molecular formula	Activity	Remarks & references
Halogenated hydrocarbons	α, α, α, α′, α′, α′-hexa-chloro-p-xylene $C_8H_4Cl_6$	Blood schizontocidal In Rane test (Osdene et al 1967) *P. berghei*/mouse active at 160 mg/kg, curative at 640 mg/kg. SD_{90} in *P. berghei*/mouse (6 days p.o. as from infection) 36 mg/kg (quinine 74.5 mg/kg). Also active in *P. cynomolgi*/*Macaca mulatta*	Activity of the α, α, α, α′, α′, α′-4, 6-octachloro-m-xylene analogue independent of resistance to sulfones, cycloguanil, chloroquine (*P. berghei*). Group may merit further follow-up Elslager et al (1970)
Benzophenones	3, 4-dichloro-4′-trifluoro-methyl-benzophenone guanyl-hydrazone HCl $C_{15}H_{11}Cl_2F_3N_4$-HCl	Blood schizontocidal In Rane test (Osdene et al 1967) *P. berghei*/mouse 100% curative at 80, 160, 320, 640 mg/kg	This is the most active among a series of 24 guanylhydrazones of polyhalogen-substituted benzophenones, 18 of which showed antimalarial activity Do Amaral et al (1971)
4, 7-Dioxobenzothiazoles	5-n-undecyl-6-hydroxy-4, 7-dioxobenzothiazole $C_{18}H_{25}O_3NS$	Blood schizontocidal (tissue schizontocidal?) In sporozoite-induced *P. gallinaceum* malaria in chicken 1/5 deaths at 30 mg/kg, 4/5 deaths at 120 and 240 mg/kg in prophylactic test (one-dose assay, s.c. administration)	No drug deaths at 240 mg/kg. Acts probably as antimetabolite of coenzyme Q. Group may merit follow-up Friedman et al (1973)
1-Naphthalenemethanols	6-chloro-α-(dibutylamino-methyl)-3-(3, 4-dichlorophenyl)-1-naphthalenemethanol hydrochloride $C_{26}H_{30}Cl_3NO$-HCl	Blood schizontocidal In Rane test (Osdene et al 1967) *P. berghei*/mouse 3/5 cures at 10 mg/kg; 5/5 cures at dose levels 20+ mg/kg	Most active compound among a series of 22. Activities comparable to those of 4-quinolinemethanols. No phototoxicity Gillespie et al (1975)
1, 2-Dimethoxynaphthalenes	4[(4′-amino-1′-pentyl) amino]-1, 2-dimethoxy-6-bromonaphthalene $C_{17}H_{23}BrN_2O_2$	Tissue schizontocidal Radically curative in *P. cynomolgi* sporozoite-infected *Macaca mulatta* at 10 mg/kg for 7 days (p.o.)	Not toxic at 10 mg/kg/day (7 days) in contrast to other effective members of this group. Compound was used as fumarate. Acts probably as prodrug for the corresponding o-quinone Archer et al (1980)
Naphthothiophenes	6, 8-dichloro-α-(di-n-butyl-aminomethyl)-4-naphtho [2, 1-b]-thiophene-methanol $C_{22}H_{28}Cl_3NOS$	Blood schizontocidal In Rane test (Osdene et al 1967) *P. berghei*/mouse active at 160 mg/kg; 3/5 cures at 320 mg/kg; 5/5 cures at 640 mg/kg	Br or CF_3 substitution in the 8-position yielded also active compounds; α-(N-piperidinomethyl) methanol substitution in 4 produced inactive compounds Das et al (1972)

Anthracene aminoalcohols	9-(3-n-butylamino-1-hydroxypropyl)-10-chloro-3-trifluoro-methylanthracene hydrochloride $C_{22}H_{23}NOClF_3$-HCl	Blood schizontocidal In Rane test (Osdene et al 1967) *P. berghei*/mouse significant increase of mean survival time (IMST) at 80 mg/kg and 160 mg/kg. Higher doses not tested	Another compound of this group. 9-(-2-di-n-butylamino-1-hydroxyethyl)-2, 3-dichloroanthracene-HCl was curative at 640 mg/kg in the Rane test Traxler et al (1975)
Fluorenemethanols	α-(di-n-butylaminomethyl)-2, 7-dichloro-4-fluorenemethanol hydrochloride $C_{23}H_{29}NOCl_2$-HCl	Blood schizontocidal (causal prophylactic?) In *P. berghei*/mouse model active at 20 mg/kg and fully curative at 80+ mg/kg. Causal prophylactic activity in *P. gallinaceum*/chicken at 10 mg/kg	Most active of 14 compounds in this series. Two more had blood schizontocidal activity, but at higher dose levels Atkinson & Granchelli (1974)
6-Aminoquinolines	6-(4-diethylamino-1-methyl-butylamino)-2, 4-dimethyl-5, 8-dimethoxyquinoline $C_{22}H_{35}N_3O_2$	Tissue schizontocidal (radically curative) (causal prophylactic?) In *P. cynomolgi*/*Macaca mulatta* radically curative. Primaquine index though < 0.5. Has not been tested for causal prophylactic activity	6-(4-amino-1-methylbutyl-amino)-4-methyl-5, 8-dimethoxyquinoline (WR 203 766) has causal prophylactic activity at 40 mg/kg in mice but no radical curative effect against *P. cynomolgi*. Some members of this group are also weak blood schizontocidals Fink et al (1970) Nickel et al (1977) Davidson et al (1981)
Dihydroacridinediones	7-chloro-10-hydroxy-3(4-trifluoromethylphenyl)-3, 4-dihydroacridine-1, 9-(2H, 10H)-dione Floxacrine, HOC 991, WR 233 602 $C_{20}H_{12}NO_3ClF_3$	Tissue schizontocidal Causal prophylactic Highly active as blood schizontocidal. CD_{50}/CD_{90} in *P. berghei*/mouse 4.3/6.7 mg/kg p.o., and 1.7/3.6 mg/kg s.c. Also highly active in *P. cynomolgi*. Effect independent from resistance to chloroquine. Causal prophylactic action in *P. berghei* and *P. cynomolgi*	Compound was highly advanced in secondary and tertiary screens but clinical development was not pursued as it causes endarteritis in dogs. Other members of group are of interest and merit follow-up Raether & Fink (1979) Schmidt (1979c) Davidson et al (1981) Raether et al (1981)
Piperazine derivatives	1-(4-methoxycinnamoyl)-4-[5-(4-bromophenyl)-4-oxo-2-oxazolin-2-yl] piperazine	Blood schizontocidal In Rane test (Osdene et al 1967) *P. berghei*/mouse active at doses of 80+ mg/kg	This is the most active of 69 compounds of this group. The 4-chlorophenyl analogue showed similar activity Herrin et al (1975)

Table 51.6 (*contd*)

Group	Name & molecular formula	Activity	Remarks & references
Thioguanines	2-amino-6-[S-propyl(n)] thiopurine $C_8H_{12}N_5S$	Blood schizontocidal At 100 mg/kg highly active against chloroquine-resistant *P. berghei* in mice. Activity was higher than that of thioguanine	This is the most active out of a series of 14 compounds (2, 6, 9-substituted thioguanines). 2-amino-6-(S-acetonyl) thiopurine showed similar activity L Q Li et al (1982)
Adenosine derivatives (mycostatics)	sinefungin 6, 9-Diamino-1-(6-amino-9H-purin-9-yl)-1, 5, 6, 7, 8, 9-hexadeoxy-ß-D-ribodecofuranuronic acid $C_{15}H_{23}N_7O_5$	Blood schizontocidal In vitro (*P. falciparum*) highly active. $IC_{50} < 0.2 \times 10^{-6}$ M, growth arrest most marked at trophozoite stage. Single dose treatment in *P. vinckei petteri* ineffective at approximately 50 mg/kg	Shares antimalarial activity with 3-deazaadenosine and 5'-deoxy-5'-(isobutylthio)-3 deazaadenosine which are methylation inhibitors. Marked inhibition also in trypanosomes and leishmania Trager et al (1980) Lederer (1983)
Arylpteridines	2,4,7-triamino-6-o-tolyl-pteridine $C_{13}H_{13}N_7$	Blood schizontocidal (causal prophylactic?) Probably antifol In *P. berghei*/mouse (Rane test, Osdene et al 1967) at 160 mg/kg 7 × as effective as quinine sulfate, and 1.5 × as effective as chloroquine diphosphate	LD_{50} mouse p.o. > 5071 mg/kg, i.p. 3270 mg/kg; in rat p.o. 1800 mg/kg, i.p. 1260 mg/kg. Relatively toxic in dogs. Group may merit follow-up Osdene et al (1967)
Tetrahydrofurans	2-(p-chlorophenyl)-2-(4-piperidyl)-tetrahydrofuran BA 41 799 WR 93 133 $C_{15}H_{19}NOCl$	Causal prophylactic Blood schizontocidal In *P. berghei*/mouse blood schizontocidal CD_{50} 80 mg/kg s.c., causal prophylactic at 40 mg/kg p.o. and s.c. Active also against chloroquine-resistant *P.berghei*. No radical curative activity in *P. cynomolgi/Macaca mulatta*	May act through inhibition of tetrahydrofolate dehydrogenase. Various analogues (WR 190 729, WR 179 305, WR 199 334) exhibit similar activity, causal prophylactic effect being most marked with compounds having an 'opened' furan structure Peters (1970b) Cheng (1971)
Polyheterocyclic compounds (antibiotics)	erythromycin $C_{37}H_{67}O_{13}N$	Blood schizontocidal In 4-day test (Peters 1965), *P. berghei*/mouse, ED_{90} with base s.c. 480 mg/kg for chloroquine- and quinine-resistant RC isolate, 500 mg/kg for chloroquine-resistant, quinine-sensitive NS isolate and for chloroquine- and quinine-sensitive N isolate. ED_{90} for stearate p.o. 430 mg/kg	Marked potentiation of chloroquine effect under erythromycin 300 mg/kg in RC isolate, reducing chloroquine ED_{90} from > 400 to 1.25 mg/kg. Erythromycin acts through inhibition of protein synthesis. Synergism with other compounds merits exploration Warhurst et al (1972, 1976)

N isolate. ED_{90} for stearate p.o. 430 mg/kg

Cyclic peptides (cytostatics)	cyclosporin A [R−[R*, R* −(E)]]-cyclic (L-alanyl-D-alanyl-N-methyl-L-leucyl-N-methyl-L-leucyl-N-methyl-L-valyl-hydroxy-N, 4-dimethyl-L-2-amino-6-octenoyl-L-α-amino-butyryl-N-methylglycyl-N-methyl-L-leucyl-L-valyl-N-methyl-L-leucyl) $C_{62}H_{112}N_{11}O_{12}$	Blood schizontocidal 25 mg/kg p.o. for 4 days in *P. berghei*/mouse is active, late treatment more effective than early treatment. Similar results in *P. yoelii yoelii*. In *P. chabaudi*/mouse cylosporin A apparently synergistic with pyrimethamine	In vitro studies with *P. falciparum* showed complete inhibition at 8.32×10^{-9} M, and still marked inhibition at 0.832×10^{-9} M. Contrary to *P. chabaudi* no reduction of sensitivity after exposure to cyclosporin A Thommen-Scott (1981) Nickell et al (1982)
Chinoid chromopeptides (antibiotics/cytostatics)	actinomycin D N, N′-[(2-amino-4, 6-dimethyl-3-oxo-3H-phenoxazine-1, 9-diyl)bis [carbonylimino[2-(1-hydroxyethyl)-1-oxo-2, 1-ethanediyl] imino[2-(1-methyl-ethyl)-1-oxo-2, 1-ethanediyl]-1, 2-pyrrolidinediylcarbonyl (methylimino)(1-oxo-2, 1-ethanediyl)]]bis/N-methyl-L-valine] di-ξ-lactone $C_{62}H_{82}N_{12}O_{16}$	Blood schizontocidal (tissue schizontocidal?) Actinomycin D at single i.p. dose of 0.01 mg/kg reduced *P. vinckei* parasitaemia in mice by 58%, not significantly different from the effect of 2 × 0.01 mg/kg, but higher than that of chloroquine at 1 mg/kg	Actinomycin D has marked cytostatic activity. It seems to interfere with RNA synthesis. The single dose LD_{10} of actinomycin D in mice: approximately 0.15–0.25 mg/kg. Another cytostatic agent, cyclophosphamide has also been shown to have antimalarial activity Fink & Goldenberg (1969)

the new compounds reached the duration of activity of 4',4''' sulfonylbisacetanilide (acedapsone, DADDS) in both the *P. berghei* and *Mycobacterium leprae*/mouse models.

These examples show the uncertainty associated with the search for new antimalarial drugs. Upon the discovery of an active lead compound, a substantial effort in the synthesis and evaluation of analogues in primary, secondary and advanced screens may have to be made before the limitations of the new candidate drugs become known. This was particularly pronounced before the introduction of an in vitro drug screening system based on the use of various isolates of *P. falciparum* and the incorporation of radiolabelled hypoxanthine or isoleucine (Desjardins et al 1979b). This technique anticipates, to some extent, the results which were previously only available from the relatively late simian screens. An example of this approach is the in vitro assessment of the activity of a series of 2-acetylpyridine thiosemicarbazones (Lambros et al 1982) which provided an insight into the structure/activity relationships of this group of compounds. In particular, their activity was independent of chloroquine-resistance. In vivo studies in the *P. berghei*/mouse system were less encouraging. It has to be realized that the in vitro screening model also has obvious limitations, especially with regard to prodrugs.

The group of 2-acetylpyridine thiosemicarbazones continues to be explored with promising results (Klayman et al 1983, 1984b; Scovill et al 1984). Other chemical groups of interest are 4-amino-substituted pyrocatechols (Schmidt 1983b; Schmidt et al 1985), 6-aminoquinolines (Dann et al 1982), 4-arylamino-tert-butylaminomethylphenols (F L Li et al 1982), (2-substituted styryl)-4-aminopyridines (Zhang et al 1983) and α-alkylaminomethyl-1,6-dichloro-4-fluorenemethanols (Zhao et al 1982).

Besides the compounds listed in Table 51.6 there are various other groups which deserve attention as potential antimalarial drugs or as principles of structure/activity relationships which may ultimately serve the rational, lead-directed synthesis of novel candidate substances. In the former group belong antibiotics, cytostatics, alkaloids and other plant-derived substances (see also section on mode of action, p. 1578); in the latter belong, among others, aromatic chelators and relatively simple molecules with antimalarial activity.

Tetracycline has become a drug which is widely used, in association with quinine, for the treatment of infections with multiresistant *P. falciparum*. Other drugs of the same group such as doxycycline and minocycline (Fig. 51.41 & 51.42) are known to be potent, albeit slowly acting, antimalarial compounds, but their wider clinical evaluation in this area has only recently started.

Erythromycin, if taken alone, has an antimalarial effect inferior to that of tetracycline. Erythromycin at 2 × 150 mg/kg or 1 × 300 mg/kg body weight daily for 15 days cleared patent infections of *P. knowlesi* in *Macaca mulatta* but was followed by recrudescence (Warhurst et al 1983). Warhurst (1977) observed a potentiation between erythromycin and chloroquine against chloroquine-resistant *P. berghei*. This potentiation was not seen in chloroquine-sensitive lines. In eastern Thailand combinations of chloroquine and erythromycin as well as of chloroquine and tetracycline were studied in the treatment of uncomplicated, highly chloroquine-resistant falciparum malaria (Phillips et al 1984). The results were disappointing. Since it had been suggested that the erythromycin doses in these trials had been inadequate, another study was carried out with large doses of erythromycin associated with standard dose regimens of chloroquine and quinine (Pang et al 1985). Although the effect of the

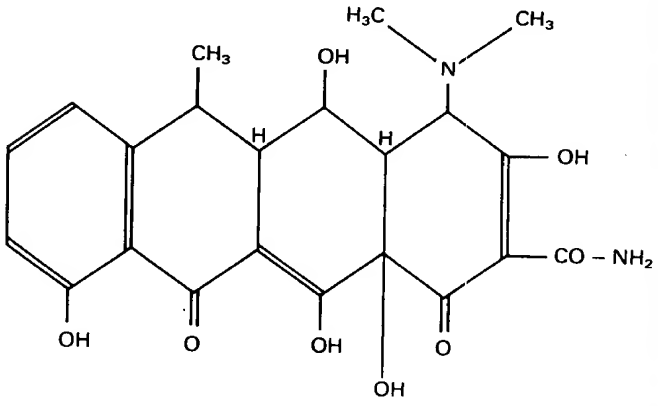


Fig. 51.41 Doxycycline

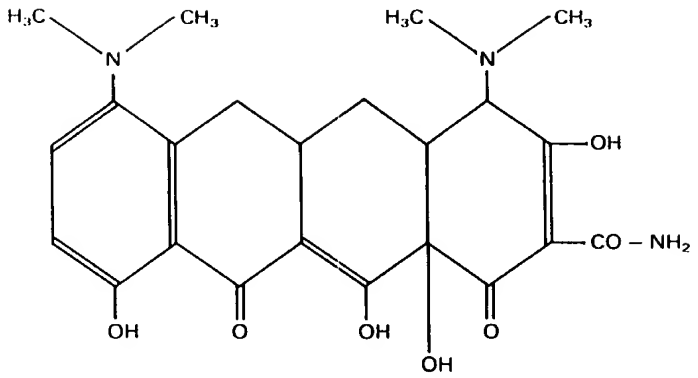


Fig. 51.42 Minocycline

quinine-erythromycin association (2 S and 8 RI) was better than that of the chloroquine-erythromycin association (6 RII and 4 RIII), the low overall cure rate makes it an unacceptable regimen with high potential risk to the patients.

Salinomycin, an ionophorous polyether antibiotic, was found to exert significant activity against *P. falciparum* and *P. berghei* in vitro and *P. berghei* in rats after single s.c. doses of 20 mg/kg or repeated s.c. administration of lower doses such as 3 × 5 mg/kg, 5 × 2.5 mg/kg or 5 × 1.5 mg/kg (Mehlhorn et al 1984; Raether & Mehlhorn 1984; Raether et al 1984). Oral administration failed to produce an antiplasmodial effect. Lasacolid, a similar ionophorous antibiotic, was highly active against *P. falciparum* and *P. berghei* when in complex with monovalent cations such as K^+ or Na^+ but was inactive when in complex with divalent cations. e.g. Ca^{++} Although polyether compounds are generally toxic they may provide interesting leads for new antimalarial drug principles. Amphotericin B and two other antifungal agents, ketoconazole and miconazole, were strong inhibitors of *P. falciparum* in vitro at clinically achievable (non-toxic) drug concentrations (Pfaller & Krogstad 1981), both with chloroquine-sensitive and -resistant isolates.

Cyclosporin A, an undecapeptide and metabolite of the fungus *Tolypocladium inflatum* was found to possess a potent effect against *P. berghei* and *P. yoelii* in mice and *P. falciparum* in vitro (Nickell et al 1982). Cyclosporin A, which was earlier recognized as an immunosuppressive compound with a selective activity against T-lymphocytes, is used for controlling graft rejection, mainly in connection with renal allografts (for review see Kahan 1984). However, it has become clear that the spectrum of activity of the compound is considerably wider. Preliminary observations on its use for the treatment of severe and complicated malaria (Trin Kim Anh & C Jerusalem, personal communication) suggest a high therapeutic potential in association with the usual management of such cases, including the administration of quinine by intravenous infusion.

Among the plant substances, Yingzhaosu A and to a lesser extent Yingzhaosu B (Fig. 51.43 & 51.44) from *Artabotrys uncinatus* or *hexapetalus* (Liang et al 1979a, b) have shown a significant antimalarial effect in the *P. berghei*/mouse model;

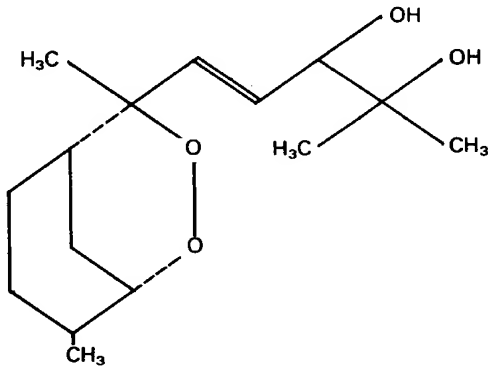


Fig. 51.43 Yingzhaosu A

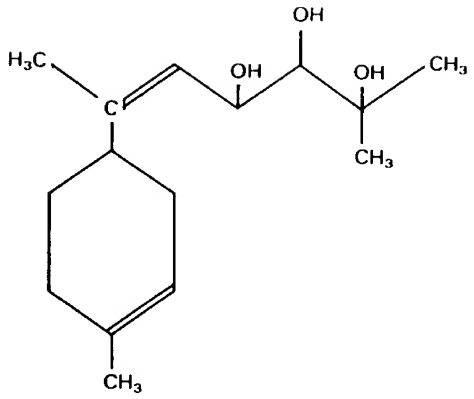


Fig. 51.44 Yingzhaosu B

clinical studies with Yingzhaosu in a small number of patients indicate a satisfactory response in human malaria (Committee on Scholarly Communication with the People's Republic of China 1979). Yingzhaosu A contains a peroxide bridge similar to that in Qinghaosu and it is believed that this structure is responsible for the antimalarial effect. Unfortunately, it seems that Yingzhaosu A is a relatively unstable compound and this poses problems, particularly during its extraction from plant material.

Another group of plant substances, now under intensive investigation for their antitumour activities, are the quassinoids, i.e. substances derived from plants of the Simaroubaceae family (see also section on mode of action, p. 1579). Trager & Polonsky (1981) found that Simalikalactone D completely inhibited *P. falciparum* in vitro at 0.002 μg/ml (approximately 4.5×10^{-9}M), whereas glaucarubinone and soularubinone were fully effective at 0.006 μg/ml (approximately 14×10^{-9}M). Simaloride and chaparrinone showed little activity even at higher doses. These authors stated that the concentrations for in vitro growth inhibition of malaria parasites were considerably lower than those for inhibition of tumour growth in vitro and suggested therefore that the therapeutic index in vitro might be better for antimalarial as opposed to antitumour activity. Such observations deserve attention since certain quassinoids are currently undergoing clinical phase I and II studies as anticancer agents which may yield important information on the tolerability and pharmacokinetics of these compounds.

Aromatic chelators such as 2-mercaptopyridine-N-oxide, 8-hydroxyquinoline and other quinoline derivatives were found to exert intensive growth inhibition of *P. falciparum* in vitro which is apparently correlated with their chelating activity (Scheibel & Adler 1980, 1981, 1982). Since some of these compounds are already being therapeutically used for the treatment of other diseases, it should be possible to evaluate potential antimalarial activity in vivo without undue delay. In this connection it is interesting to note that another chelating agent, tetraethylthiuram disulfide (antabuse) is a potent inhibitor of the growth of *P. falciparum* in vitro (Scheibel et al 1979). This compound which is used for the treatment of alcohol abuse by aversion therapy obviously interferes with the glycolysis of plasmodia without apparent effect on the glycolysis of normal erythrocytes.

Clopidol (WR 61 112) and an analogue, WR 156 949 (Fig. 51.30 & 51.45), exert a causal prophylactic effect in the *P. berghei*/mouse model with a causal prophylactic ED_{50} of 160 mg/kg (Davidson et al 1981). Although these data would not justify a follow-up of clopidol and WR 156 949 themselves, they may provide promising leads for the synthesis of more active analogues.

While the primary testing of the individual candidate compounds may yield indications of antimalarial activity and provide even a comparison between the efficacy of the oral and s.c. routes, a low activity should not essentially preclude further evaluation. Synthesis of analogues is not

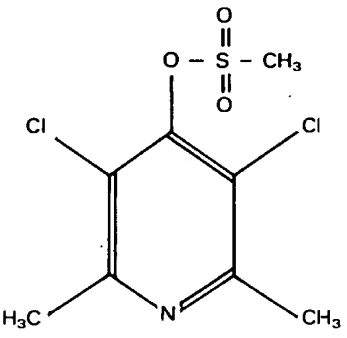


Fig. 51.45 WR 156 949

the only means of eventually arriving at more potent compounds. In advanced preclinical development it may be possible to optimize the activity by specific galenic formulation or to enhance it through appropriate combination partners. Other ways, such as polymerization, may also exist; however some of these have been forgotten and some may not have been sufficiently explored as in the example of a sulfapyridine-formaldehyde copolymer which proved to be curative in the *P. berghei*/mouse model at 80 mg/kg, whereas 640 mg/kg sulfapyridine were required for the same effect (Donaruma & Razzano 1966).

An overview of recent research on the chemotherapy of malaria yields a less gloomy picture than that of the early 1970s. There are now several compounds nearing completion of the preclinical development phase, and others that offer promise for the future. Moreover, novel approaches in the areas of lead-directed synthesis, targeting and galenic formulation may yield highly active antimalarial compounds the need for which is obvious in the presence of malaria parasites capable of producing drug resistance.

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EXHIBIT 2

Exhibit 2

Comparison of oral artemether and mefloquine in acute uncomplicated falciparum malaria

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Plasmodium falciparum malaria in Thailand is highly resistant to available antimalarials, and alternative drugs are needed urgently. Artemether is effective against falciparum malaria but associated with a high recrudescence rate. The proper dosage regimen remains to be defined. We have done a clinical trial comparing mefloquine 1250 mg in divided doses with oral artemether at 700 mg total dose given over 5 days in acute uncomplicated falciparum malaria.

46 patients, admitted to the Bangkok Hospital for Tropical Diseases, were randomised to receive either mefloquine (12) or artemether (34). Hospital follow-up was 28 days for the artemether group and 42 days for the mefloquine group. Oral artemether gave a significantly faster parasite clearance time than mefloquine (30 *vs* 64 h), and a significantly better cure rate (97 *vs* 64%) with fewer episodes of dizziness and vomiting.

Oral artemether at 700 mg given over 5 days is effective and well tolerated. The cure rate with this regimen is higher than that reported by previous studies with 600 mg intramuscular artemether given over 5 days. Oral artemether can be considered as an alternative drug for multiple-drug-resistant falciparum malaria.

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Introduction

Plasmodium falciparum in Thailand is highly resistant to chloroquine and sulfadoxine-pyrimethamine[1] and the organism is showing increasing resistance to the alternative antimalarials quinine[2] and mefloquine.[3-6] Mefloquine 1250 mg, either in a single dose or two divided doses, has a cure rate of 80–84%,[4-6] but the side-effects, particularly vomiting, may result in lower drug concentrations and consequent treatment failure.[4,5] Alternative drugs are needed urgently.

Artemether is an effective antimalarial drug with a rapid onset of action that destroys asexual parasites at an early stage of development. The potency of this drug has been shown in clinical trials in China and Burma.[7-9] Artemether clears parasites rapidly with virtually no side-effects. Artemether is, however, associated with a high rate of recrudescence that varies with duration of treatment and the total dose given. The recommended dose of artemether has been 600 mg given over 5 days, but we have found the cure rate to be only 90% with intramuscular artemether at this dose.[10] There are no reports on the efficacy of oral artemether in multiple-drug-resistant falciparum malaria, and the proper dosage regimen of artemether for the treatment of this condition remains to be decided. Based on the efficacy of oral artesunate,[6,10,11] the duration of treatment with artemether should be at least 5 days and the dose needs to be above 600 mg to achieve a cure rate approaching 100%. We report the findings of a clinical trial that compared oral artemether with mefloquine (the first-line drug for falciparum malaria in Thailand) for the treatment of multiple-drug-resistant falciparum malaria.

Patients and methods

Patients recruited to the study were 46 adult men, aged 15–50 years and weighing 45–65 kg, with acute uncomplicated falciparum malaria (asexual-form parasitaemia of less than 5%, with no complications),[12] no history of liver or kidney diseases, and no history of taking antimalarials for this episode of illness. Written informed consent for participation in the study was obtained from all patients. The study was approved by the ethics committee of the Faculty of Tropical Medicine, Mahidol University, Bangkok.

Patients' previous history of treatment with antimalarials was recorded. Before treatment, a blood smear was taken for malaria parasite identification and 3 ml venous blood was drawn for assay of baseline drug concentrations of mefloquine and quinine. All patients were admitted to the Hospital for Tropical Diseases, Bangkok. The hospital admission was for 28 days for patients who received artemether and for 42 days for those who received mefloquine.

Patients were allocated randomly (open randomisation), with a 3 to 1 probability in favour of artemether, to either oral mefloquine 750 mg initial dose followed by 500 mg 6 h later, or oral artemether 200 mg initial dose followed by 100 mg 6 h later then 100 mg daily for 4 days. Both drugs were given with a glass of water under supervision. More patients were recruited into the artemether group because this is the first trial of oral artemether and the cure rate of mefloquine is well established since it is used extensively in Thailand. The sample sizes were chosen to detect a 35% better cure rate for artemether than for mefloquine with 95% confidence based on the cure rate of mefloquine in a previous study (T. Harinasuta, unpublished observations).

Patients who failed to respond to treatment were given quinine sulphate 600 mg every 8 h and tetracycline 250 mg four times per day for 7 days. Patients with vivax malaria during follow-up were treated with chloroquine 150 mg (base) to suppress the symptoms, and a full course of treatment was given on discharge.

Parasite identification was done on thick and thin peripheral blood smears stained with Field's stain, and parasite counts were reported per 100 red blood cells or per 200 white blood cells. Blood smears were examined every 6 h until parasitaemia fell below the level of microscopic detection in a thick smear, then twice daily until day 28 for the artemether group and until 42 days for the mefloquine group. The 42-day follow-up for patients given mefloquine is necessary because of the long half-life of this drug; a follow-up of only 28 days could have underestimated the recrudescence rate by as much as 10% (unpublished observation). With drugs that have shorter half-lives, such as quinine and the artemisinin group of compounds, most recrudescence occurs within 4 weeks of treatment.[1,10,11]

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TABLE I—PATIENTS' ADMISSION CLINICAL AND LABORATORY DATA

Mean (range)	Artemether (n = 34)	Mefloquine (n = 12)
Age (yr)	24 (17–48)	26 (17–48)
Weight (kg)	55 (45–65)	52 (45–61)
Temperature (°C)	38·4 (37·5–40·3)	38·1 (37·5–39·5)
Packed cell volume (%)	37 (26–51)	36 (33–45)
White blood cells (/μl)	6644 (4200–13 500)	6111 (2600–14 090)
Parasitaemia (/μl)*	13 490 (4220–149 260)	23 438 (3900–142 560)

*Given as geometric mean (range).

Complete blood count and blood biochemistry tests were done on admission and on days 2, 4, and 7, and then weekly until days 28 or 42 depending on the treatment group. Electrocardiographs were recorded daily for 7 days then weekly for the rest of the follow-up period. Since it has been shown that some cases of failed mefloquine treatment are associated with inadequate blood concentrations of drug—ie, not true resistance—we measured mefloquine concentrations in all patients who received this drug 6, 12, and 24 h after treatment, then daily for 7 days and weekly until discharge.

Adverse reactions during the study period were recorded three times per day during the first week then daily until discharge. All abnormalities that may have been attributable to artemether or mefloquine were recorded.

Patients in the artemether and mefloquine groups were included for assessment of treatment efficacy if they had completed 28 days or 42 days, respectively, of follow-up. Efficacy and side-effects were compared between the two therapeutic regimens. The parameters used in determination of outcome included fever and parasite clearance times, the rate of treatment failure (RI, RII, or RIII),[13] and the occurrence of adverse effects. Comparison of data from the two treatment groups was by the Mann-Whitney U test. Pretreatment parasitaemia was stratified into three levels (< 10 000, 10 000–100 000, and > 100 000 parasites/μl), the Mantel-Haenszel test was used to test for differences in proportions of patients in each treatment group, and differences between treatments in parasite clearance times were then tested by analysis of variance. Levels of statistical significance between treatment groups were calculated with Fisher's exact test (two tailed) for proportion and the Mann-Whitney U test for other outcome variables.

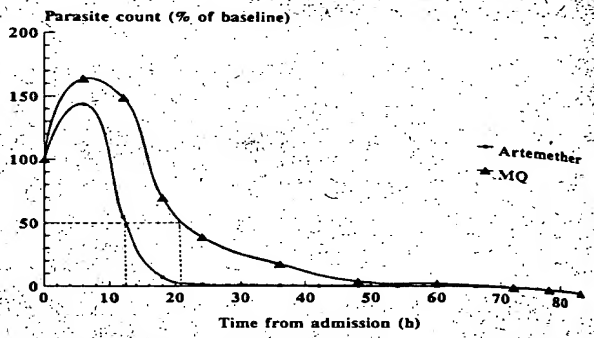
Results

Of the 46 male patients with falciparum malaria included in the study, 12 were treated with mefloquine and 34 with oral artemether. Admission clinical and laboratory data were similar in the two groups (table I). Levels of parasitaemia at admission were not statistically different between treatment groups (p = 0·113, by the Mantel-Haenszel test after stratification of admission parasitaemia into three levels). All patients presented with acute symptoms of malaria.

TABLE II—THERAPEUTIC RESPONSES

—	Artemether (n = 34)	Mefloquine (n = 12)
Mean (range) PCT (h)	34 (18–84)	64 (40–207)*
Mean (range) FCT (h)	30 (4–108)	27 (2–107)
Response (number of patients)†		
Cure	29	7
RI	1	2
RII	0	2
RIII	0	0
Cure/RI (follow-up < 28 d)	4	1
Cure rate at 28 days	97%	73%
Cure rate at 42 days	Not done	64%‡
Vivax malaria	9 (days 14–23)	1 (day 52)

*p = 0·00006 and ‡p = 0·014 compared with artemether. †Cure = no reappearance of parasite within 28 days for artemether and 42 days for mefloquine; RI = parasitaemia disappears but reappears again within 28 or 42 days; RII = decrease of parasitaemia but parasites never disappear from the peripheral blood; RIII = no pronounced decrease or increase in parasitaemia 48 h after treatment.



Mean parasite clearance curve.
MQ = mefloquine.

Parasites did not clear from the blood of 2 patients in the mefloquine group, although there was a decrease in parasitaemia; thus, these patients were classified as having an RII response. The other 10 patients in the mefloquine group had a good initial response with mean parasite clearance times (PCTs; the time taken for the parasite count to fall below the level of microscopic detection) and fever clearance times (FCTs; the time taken for the temperature to return to normal—ie, below 37·7°C—and remain at that value for at least 24 h) of 64 h and 27 h, respectively (table II). PCT was not significantly different among patients presenting with low or high pretreatment parasitaemia. 1 patient left the hospital without parasitaemia on day 7, therefore 11 patients were included in the assessment of efficacy. 2 patients had reappearance of parasitaemia on days 20 and 31 (RI response). Cure rates with mefloquine were 73% and 64% for evaluations at 28 days and 42 days, respectively.

All patients in the artemether group had a rapid initial response with mean PCTs and FCTs of 34 h and 30 h, respectively. There was no difference in PCT among patients with low and high pretreatment parasitaemia. In all artemether-treated patients parasites cleared rapidly from peripheral blood (figure). 4 patients did not complete the 28-day follow-up period, but all left the hospital without parasitaemia—2 on day 7 and the other 2 on days 19 and 21. The cure rate was therefore based on 30 patients and calculated to be 97% (95% CI 83–99·9%).

PCT was significantly faster in the artemether group than in the mefloquine group (p = 0·00006). At all three levels of pretreatment parasitaemia, artemether-treated patients had significantly quicker parasite clearance than mefloquine-treated patients (p = 0·00001, 0·0002, and 0·00001 for pretreatment parasitaemia of < 10 000, 10 000–100 000, and > 100 000 parasites/μl, respectively). The estimated mean 50% PCTs were 12 h for artemether and 21 h for

TABLE III—NUMBER *(%)* OF PATIENTS WITH SIDE-EFFECTS

—	Artemether (n = 34)	Mefloquine (n = 12)
Nausea	2 *(6)*	3 *(25)*
Vomiting	0	4 *(33)**
Dizziness	1 *(3)*	4 *(33)*†
Abdominal pain	3 *(9)*	1 *(8)*
Diarrhoea	1 *(3)*	1 *(8)*
Bradycardia	10 *(29)*	5 *(42)*

*p = 0·003 and †p = 0·013 compared with artemether.

mefloquine. There was no difference between treatment groups in FCT (table II). Cure rate was significantly better for patients treated with artemether than for those treated with mefloquine ($p = 0.014$).

Side-effects were mild and self-limiting in both treatment groups (table III). Vomiting and dizziness were found more often with mefloquine, but these symptoms were transient and required no specific treatment. Bradycardia was found in 5 patients who took mefloquine and 10 who took artemether, and it occurred most often between days 2 and 7. No other arrhythmias were noted. There were no notable drug-related blood profile and biochemical changes during the course of follow-up in either treatment group. Concentrations of mefloquine in blood were the same for patients with sensitive and resistant responses to treatment. 1 patient who was treated with mefloquine had *Plasmodium vivax* in his peripheral blood on day 52. 9 patients who were treated with artemether developed vivax malaria between days 14 and 23.

Discussion

In this comparative clinical trial, oral artemether was an effective alternative to mefloquine in the treatment of multiple-drug-resistant faciparum malaria. Artemether gave more rapid reduction of parasitaemia than did mefloquine, cure rate was significantly better with artemether, and there were fewer reports of dizziness and vomiting. Use of the artemisinin group of compounds has been associated with high recrudescence rates.[6-8,10,11] At a maximum dose of artemether of 600 mg given over at most 5 days, the recrudescence rate was about 10% in uncomplicated falciparum malaria and higher in severe falciparum malaria.[9,14] Increasing the dose of artemisinin compounds did not correlate with rapidity of parasite clearance but did correlate with cure rate.[15] When we increased the dose of artemether to 700 mg over 5 days, PCT and FCT were similar to all previous studies with intramuscular artemether at different dosage regimens, but cure rate was higher than that found with previous regimens.[8-10] Adjustment to an appropriate dosage regimen of artemether is difficult because of limited pharmacokinetic information. In addition, the bioavailability of oral artemether is not known. Dosage regimens are therefore mainly empirical. The 600 mg dose may not be suitable in areas where multiple-drug resistance is common because it is associated with high recrudescence rates,[8-10] but this dose may be adequate in areas with less drug-resistant parasites.[7,16]

In Thailand, the second-line drug regimen for treatment of falciparum malaria is quinine plus tetracycline for 7 days. The cure rate for quinine/tetracycline in 1989 was 93%,[4] but the long course of treatment causes problems with compliance. The combination of artesunate 600 mg given over 5 days followed by mefloquine 1250 mg in two divided doses has been shown to be highly effective with a cure rate of 100%.[6] This cure rate was evaluated 23 days after mefloquine administration, but some patients with recrudescent infection may have been missed because the long half-life of mefloquine means that recrudescence can occur later than 4 weeks. In the present study, cure rate was 73% when the mefloquine group was evaluated 28 days after treatment, but dropped to 64% with evaluation at 42 days. In addition to the uncertain cure rate with the artesunate and mefloquine combination, the cost of this treatment would be high, and the risk of side-effects with combination therapy is certain to be higher than with monotherapy. The period of combination treatment is, at 6 days, rather long for compliance reasons, particularly since mefloquine is given on the last day of treatment when patients are already free from malaria symptoms. Use of the artesunate plus mefloquine combination in an area where resistance to mefloquine already exists is another factor that needs careful consideration.

Although the 97% cure rate of oral artemether is not entirely satisfactory, since the aim of treatment is to achieve 100% cure, this treatment is still likely to be more acceptable to patients than the two above-mentioned combination regimens. It may be possible to adjust the dosage regimen of oral artemether to achieve 100% cure when more information on the oral bioavailability and pharmacokinetics of artemether and its metabolites becomes available.

Vivax malaria occurred sooner in the artemether-treated group than in the mefloquine group, a finding that is in agreement with several previous studies.[8,10,11,17,18] Rapid elimination of artemether may be responsible for the early appearance of *P vivax*. In mefloquine-treated patients, *P vivax* was not reported until at least 4 weeks after treatment,[19] a finding that may be associated with the long half-life of this drug. Our findings, and those of others, suggest that neither artemether nor mefloquine is effective against the intrahepatic stage of *P vivax*. Primaquine is thus required for radical cure.

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Protection against bradykinin-induced bronchoconstriction in asthmatic patients by neurokinin receptor antagonist

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Axon reflex mechanisms may be involved in the pathogenesis of asthma, but there has been no direct evidence that endogenous tachykinins cause bronchoconstriction in asthmatic subjects. We have studied the effect of a tachykinin receptor antagonist (FK-224) on bronchoconstriction induced by inhalation of bradykinin in asthmatic patients.

In a double-blind, placebo-controlled, crossover trial, ten subjects with stable asthma were given FK-224 (4 mg) or placebo by inhalation 20 min before challenge with bradykinin (0–1250 μg/ml, five breaths of each concentration) given with 5 min intervals. Bradykinin caused dose-dependent bronchoconstriction in all subjects. FK-224 significantly opposed the bronchoconstrictor effect; the geometric mean of the cumulative concentration required to elicit a 35% fall in specific airway conductance was 5·3 μg/ml after placebo and 40 μg/ml after FK-224 ($p<0·001$). Inhalation of bradykinin caused coughing in three subjects, which was inhibited by FK-224 in all three.

Antagonism of the tachykinin receptor by FK-224 greatly inhibited both bronchoconstriction and coughing induced by bradykinin in asthmatic patients, suggesting that tachykinin release from the airway sensory nerves is involved in responses to bradykinin. Tachykinin receptor antagonists may be useful in the treatment of asthma.

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Introduction

Asthma is classified as an inflammatory disease[1] because, even in its mildest form, there are inflammatory changes in the asthmatic airways.[2,3] There is now much evidence that sensory neuropeptides are involved in these inflammatory responses.[4] Neurogenic inflammation is thought to be caused by the antidromic release of neuropeptides from vagal afferent C-fibres by way of an axon reflex,[5] as occurs in the skin, eyes, gastrointestinal tract, and bladder.[6,7] In experimental studies, sensory neuropeptides, especially tachykinins such as substance P and neurokinin A, can bring about many of the pathophysiological features of asthma. Neurokinin A is a very potent constrictor of human airways in vitro,[8] and substance P causes mucus secretion in the airways.[9] However, no axon reflex mechanism, brought about by endogenous tachykinin, has been demonstrated in human airways in vivo.

The involvement of kinins in inflammatory disease such as asthma has been the subject of much speculation.[10] Inhalation of bradykinin causes bronchoconstriction in asthmatic patients but not in normal subjects.[11] Since the bradykinin-induced bronchoconstriction is partly opposed by anticholinergic agents and since bradykinin is only a weak constrictor of human airways in vitro, it has been suggested that the bronchoconstrictor response is partly mediated by a neural reflex. Bradykinin stimulates vagal afferent C fibres and causes bronchoconstriction in dogs.[12] In guinea-pig airways, bradykinin causes a bronchoconstrictor response by way of cholinergic and sensory-nerve-mediated mechanisms.[13] Bradykinin-induced bronchoconstriction in human airways may therefore be due partly to tachykinin released from sensory nerve terminals via axon reflex mechanisms.

We have produced a new cyclopeptide tachykinin receptor antagonist FK-224 (fig 1).[14,15] This antagonist was produced by catalytic hydrogenation of the fermentation product isolated from *Streptomyces violaceusniger*;[14,15] it has

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